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Larval fish diversity on the Scotian Shelf

N.L. Shackell and K.T. Frank

Abstract: We examined larval fish diversity on the Scotian Shelf using data, representing 91 genera, collected during the Scotian Shelf Ichthyoplankton Program from 1978 to 1982. Two diversity indices (genus richness (GR) and Shannon's entropy (H)) were relatively lower from December to February–March and relatively higher and stable from April to September–October. Taxon composition changed seasonally. Total median log abundance ($\log_{10}$(number of individuals + 1)·1000 m^{-3}) was low from December to February, increased in March, was stable from April to June, and declined from July to October. Our results suggest that the abundance trends of most taxa were not coincident with either a spring or fall bloom of calanoid copepods. Log GR was significantly positively related to H ($r = 0.62$, $p < 0.001$, $n = 1853$). A negative exponential best described the relationship between log GR and log abundance ($R^2 = 0.77$; log GR = $1.37(1 - e^{-(1.13(\log \text{abundance}))})$, $p < 0.001$, $n = 2357$). Shannon's H was not related to log abundance in winter or in summer–fall and was negatively correlated in spring–summer ($r = -0.12$, $p = 0.003$, $n = 593$). Thus, diversity increases with abundance but the composition is dominated by relatively fewer genera at higher levels of abundance. Western – Sable Island banks had higher levels of GR and abundance in all seasons. Additional banks were diverse and productive during warmer months.

Résumé : Nous avons étudié la diversité des larves de poisson sur la plate-forme néo-écossaise avec des données couvrant 91 genres et recueillies dans le cadre du programme d'étude de l'ichthyoplancton de cette plate-forme de 1978 à 1982. Deux indices de diversité (diversité des genres (GR, pour «genus richness») et entropie de Shannon (H)) étaient relativement plus bas de décembre à février–mars et relativement plus élevés et stable d'avril à septembre–octobre. La composition par taxons changeaient saisonnièrement. Le logarithme de l'abondance totale médiane (log10(nombre d'individus + 1)·1000 m^{-3}) était bas de décembre à février, augmentait en mars, était stable d'avril à juin, et diminuait de juillet à octobre. Nos résultats laissent entendre que les tendances des abondances de la plupart des taxons n'étaient pas liées à une prolifération printanière ou automnale de copépodes calanoïdes. Le paramètre log GR était de façon significative positivement corrélé avec H ($r = 0.62$, $p < 0.001$, $n = 1853$). Une exponentielle négative décrivait le mieux la relation entre log GR et le logarithme de l'abondance ($R^2 = 0.77$; log GR = $1.37(1 - e^{-(1.13(\log \text{abondance}))})$, $p < 0.001$, $n = 2357$). Le paramètre H de Shannon n'était pas relié au logarithme de l'abondance en hiver ou dans la période été–automne et était corrélé négativement dans la période printemps–été ($r = -0.12$, $p = 0.003$, $n = 593$). Ainsi, la diversité s'accroît avec l'abondance, mais la composition est dominée par un nombre relativement plus faible de genres quand les abondances sont plus élevées. Le banc occidental et le banc de l'île de Sable présentent une diversité de genres et une abondance plus élevées en toutes saisons. D'autres bancs montraient une diversité et une productivité importantes durant les mois les plus chauds.

[Traduit par la Rédaction]

Introduction

The conservation of biological diversity has become a primary objective in various policy frameworks around the world, specifically those designed to incorporate an ecological approach to resource management (Grumbine 1997). The implementation of such an objective is proving problematic, in part because the patterns of biological diversity are not always known. The mechanisms or processes that maintain or cause changes in biological diversity, which can occur at a variety of scales, are even less clear. In this paper, we characterize broad-scale patterns of larval fish diversity and abundance as an initial contribution towards discerning how such patterns are created, maintained, or changed on the Scotian Shelf. Processes responsible for patterns of diversity can occur at a variety of scales.

We explore whether the observed patterns of diversity were related to broad-scale patterns of zooplankton abundance and (or) the climatological circulation. Larval fish assemblages have been related to hydrographic and physical oceanographic features in a wide range of systems (e.g., Frank and Leggett 1983; Cowen et al. 1993; Moser and Smith 1993). Larval assemblages have been further explained as a result of convergent spawning strategies among members of a given assemblage (e.g., Sherman et al. 1984; Doyle et al. 1993). Iles and Sinclair (1982) proposed that spawning has evolved to occur in areas that would enhance retention and that retention was positively related to survival (also see Werner et al. 1996; Shackell et al. 1999). In a review of life history strategies, Winemiller and Rose (1992) theorized that the response to seasonality in temperate zones is to spawn during a time when favorable conditions exist for survival. Cushing's related "match–mismatch" hypothe-

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sis states that fish have evolved to spawn so that the peak in post-yolk-sac larval abundance coincides with a peak in prey availability (Cushing 1975, 1995).

Although larval distribution may be a result of convergent spawning strategies, it is equally plausible that diverse strategies could result in the same distribution because certain areas or times favor survival. The common predictions of these varied hypotheses are that larvae that coincide with high food abundance and (or) occur in retention areas have a greater probability of survival. Our study gave us the opportunity to assess the generality of these predictions. If these hypotheses have general application, we would expect that larval fish diversity is greatest when and where physical retention (e.g., gyres) is highest and (or) when prey abundance is greatest.

Materials and methods

Scotian Shelf Ichthyoplankton Program (SSIP) sampling protocol

The data used in this study were collected on the Scotian Shelf from 1978 to 1982 as part of the SSIP (Fig. 1; Table 1). A primary goal of the SSIP was to collect temporal and spatial information about fish eggs and larvae and associated environmental data across the Scotian Shelf. A related goal of the SSIP was to characterize the distribution, abundance, mortality, and growth rates of various ichythyoplankton species. The survey design was based on standard sampling along a transect grid.

Sampling protocol included standardization of various methods. An oblique Bongo tow was used typically with a 333-μm-mesh net deployed for a variable time, but most typically 30 min. Flowmeters were attached to the Bongo net to determine total volume filtered. Surface temperature and bottom depth were recorded for each sample. Bottom depth represented the distance from the surface to the bottom, whereas sampling depth represented the depth at which the sample was taken. Sampling tow depths ranged from 23 to 247 m, averaging 112 m, and the ranges of sampling depth were similar among months. Samples were stored in 5% formalin and then were identified and catalogued at the Huntsman Marine Laboratory, St. Andrew's, New Brunswick, Canada. Further information on the SSIP survey design and a physiographic description of the Scotian Shelf are available in O'Boyle et al. (1984).

We extracted only larval stage data from the original SSIP data set. Initially, our intent was to conduct a species-level analysis, but we eventually opted to conduct a genus-level analysis because of several limitations of the data. First, the identification to species level within some of the more common species, e.g., *Sebastes*, is difficult during the larval stage. It is also true that the taxonomic description of many of the common species is better developed than that of rare species, e.g., *Melanogrammus aeglefinus* and *Gadus morhua*, whereas rarer species would be identified only to higher taxonomic levels. For each genus, we itemized the possible number of species known to occur in the Scotian Shelf region (Scott and Scott 1988) and concluded that the majority of genera studied herein contain one to three species (Table 2). Genus diversity is, to some extent, representative of species diversity in our study because of the small number of species within many of the genera. Finally, in the extracted data set, 31.2% of the observations had been identified to the generic level, 58.9% to the species level, and 3.6% to subfamily and higher levels and 6.3% were unidentified. A genus-level analysis allowed us to use the majority (90.1%) of the data.

Diversity and abundance indices

We examined two measures of diversity, genus richness (GR) and Shannon's entropy index (H) (Magurran 1988; Legendre and Legendre 1998). GR was measured as number of genera per 1000 m^{-3} and H was measured as

$$H = \sum_{i=1}^{q} p_i \log_{10} p_i$$

where $p_i = n_i / N$, the proportion of individuals n of genus i of the total number of individuals N in a sample, and q is the total number of genera within a sample. Evenness, or the proportional abundances of each genus within a sample, is also an aspect of diversity. Shannon's H accounts for both richness and abundance of each genus. A sample in which a given number of genera have equal abundance can be considered more diverse than the same number of genera dominated numerically by one or two genera.

We also examined abundance patterns expressed as the number of individuals per 1000 m^{-3}. Both GR and abundance were lognormally distributed and were $\log_{10}(x + 1)$ transformed for various analyses. To ensure that patterns were not a function of sampling design, we examined the relationship among number of fish genera, number of individuals, volume towed, and sampling depth. The volume towed increased with sampling depth ($r = 0.71$, $p < 0.001$, $n = 2260$); the greater the depth, the more water that was filtered. Both volume towed and sampling depth were negatively related to number of fish genera and number of individuals; most individuals were observed at depths less than 110 m. When sampling depth is held constant, the number of genera and number of individuals were not related to volume towed. The number of genera and number of individuals were only weakly related to the depth of tow ($r = -0.13$, $p < 0.001$, $n = 2257$ and $r = -0.11$, $p < 0.001$, $n = 2257$, respectively), holding tow volume constant. Given that the majority of taxa and individuals occurred in a shallower depth range, that the range of sampling depths was similar among months, and that the relationships between sampled variables and depth were weak, we assumed that our results were not an artifact of the sampling procedure.

Not all months or areas were sampled each year during the SSIP, resulting in a patchwork of samples that precluded a meaningful analysis of among-year effects for each month. It might be possible to extract a subset of the data in which the same area had been sampled during the same period each year to examine interannual variability. However, our intent was to examine broad-scale spatial and seasonal patterns. To create a data set containing information on all months of the year and over a broad spatial scale, a stepwise procedure was followed. We examined interannual variability in H and GR in each month among years. The number of years within months varied from 1 to 5. If there were no significant differences among years within a month (based on Kruskal–Wallace nonparametric analyses), the data were pooled and considered to be representative of that month. If there were significant differences within months among years, we selected those survey years that were similar and had the largest sample size and the broadest spatial sampling. The net result was a monthly time series of GR, H, and abundance representing samples from across the shelf.

Cluster analysis

One of our goals was to examine larval assemblages. Among the logistic problems associated with creating assemblages was that the distribution of sampling ranged over seasons and different regions. That is, the same taxa could occur at different locations on the shelf but in similar environments or at different times of the year. One approach would be to identify a series of separate sets of assemblages of genera within seasons and similar regions. We

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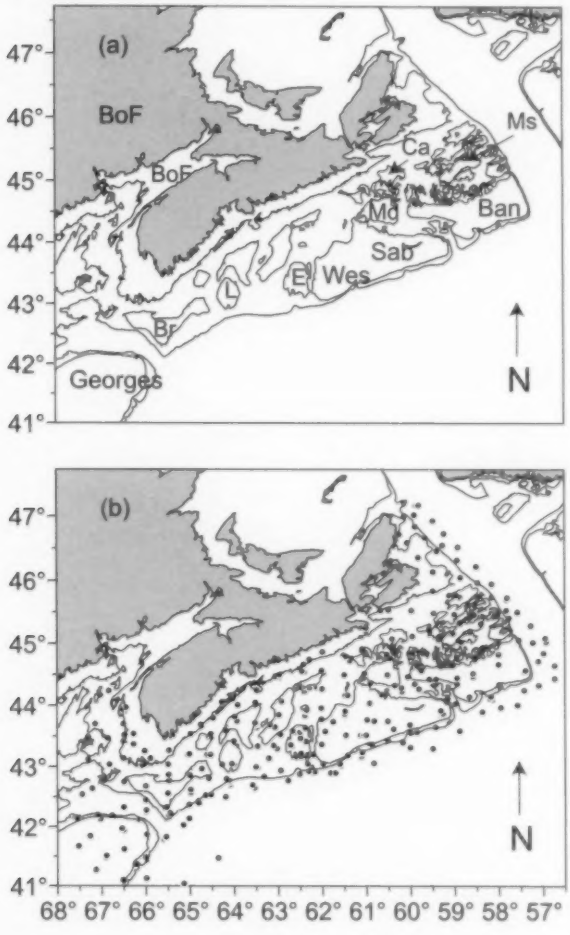
Fig. 1. (*a*) Scotian Shelf and Bay of Fundy: BoF, Bay of Fundy; Br, Browns Bank; L, LaHave Bank; E, Emerald Bank; Wes, Western Bank; Sab, Sable Island Bank; Md, Middle Bank; Ban, Banquereau; Ms, Misaine Bank; Ca, Canso Bank. (*b*) Distribution of SSIP samples from 1978 to 1982.



opted for an approach that allowed us to examine a single set of assemblages that accounted for seasonal differences among regions. We identified assemblages of genera that co-occur in the same type of habitat as characterized by temperature and bottom depth (not sampling depth). Each combination of surface temperature and bottom depth could be considered a "habitat type," and similar habitat types can co-occur across the shelf. Before clustering, we aggregated observations of each taxon by surface temperature and bottom depth, weighted by abundance. This resulted in a taxon's average surface temperature and bottom depth of occurrence. These data were then subjected to cluster analysis. Members of a cluster did not necessarily occur at the same site but in similar combinations of surface temperature and bottom depth.

We used a hierarchical (agglomerative) clustering method and Ward's method as the distance formula (Legendre and Legendre 1998). Ward's method uses the minimum variance summed over all variables as a measure of distance between two points. The groups with the smallest distance between them are joined, and then, groups are added successively using minimized distance as a criterion. We selected the number of clusters using the rate of change in the distance between clusters as a guideline. When the distance between clusters increased, we chose that as a point to select cluster groups. There was no depth information recorded for single samples of four genera, *Gonichthys*, *Gonostoma*, *Nessorhamphus*, and *Notolynchus*, so they were not included in the cluster analysis.

Table 1. Number of samples by year and month extracted from the SSIP database.

	Survey year					
Month	1978	1979	1980	1981	1982	Total
Jan.		27		87	46	160
Feb.			90	68		158
Mar.				44		44
Apr.		117				117
May		74	134		35	243
June			149			149
July			64		67	131
Aug.	199	196	150			545
Sept.	125	90	121	87		423
Oct.			118	62		180
Nov.		85	40			125
Dec.		30	53			83
Total	324	619	919	348	148	2358

Results

Genera composition

The relative frequency of occurrence of the 91 genera extracted from the SSIP database is shown in Table 2. *Merluccius*, *Sebastes*, *Urophycis*, *Glyptocephalus*, and *Ammodytes* were the most frequently encountered genera. Their relative duration of spawning, larval phase or development rate, spatial distribution, and (or) abundance may be greater than that of other genera. The distributions of abundance for each genus were highly skewed. The highest median abundance was recorded for *Merluccius*, followed by *Scophthalmus*, *Ammodytes*, *Urophycis*, and *Melanogrammus* (Table 2).

Seasonal and spatial patterns of larval fish diversity and abundance

Median log GR was relatively low from December to February, increased in March, was fairly constant from April to September, and declined in October–November (Fig. 2*a*). Shannon's H was relatively low from November to March and relatively higher from April to October (Fig. 2*b*). Total median log abundance was low from December to February, increased in March, was stable from April to June, and declined from July to October (Fig. 2).

To present the spatial patterns and relationships among the diversity indices and abundance, months were grouped into three seasons based on the temporal and spatial similarity of diversity on a monthly scale: (*i*) December–March (winter), (*ii*) April–July (spring–summer), and (*iii*) August–November (summer–fall). If relationships were significant within seasons, we grouped seasons and reported the overall relationship.

Log GR was significantly positively related to H (r = 0.62, $p < 0.001$, n = 1853) (Fig. 3*a*). A negative exponential best described the relationship between log GR and log abundance (R^2 = 0.77; log GR = $1.37(1 - e^{-(1.13)(\log \text{abundance})})$, $p <$

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Table 2. Genus, common name(s), possible number of species within genus in the Scotian Shelf area, frequency of occurrence, percentage of total count, and mean and median abundance of 91 genera.

Genus	Common name(s)	No. of species	Frequency	% of total count	Mean abundance	Median abundance
Merluccius	Offshore, silver hakes	2	768	14.24	623.35	27.66
Sebastes	Redfish	3	568	10.53	22.25	8.13
Urophycis	Longfin, red, white hakes	3	554	10.27	116.00	17.99
Glyptocephalus	Witch flounder	1	413	7.66	23.11	7.71
Ammodytes	Sandlance	2	412	7.64	235.13	22.20
Enchelyopus	Fourbeard rockling	1	274	5.08	9.32	5.16
Pleuronectes	Yellowtail flounder	1	258	4.78	66.01	12.98
Gadus	Atlantic cod	1	237	4.39	33.05	7.43
Benthosema	Lanternfish	1	180	3.34	19.22	7.38
Hippoglossoides	American plaice	1	161	2.99	24.47	9.00
Tautogolabrus	Cunner	1	154	2.86	14.02	5.79
Melanogrammus	Haddock	1	136	2.52	43.28	16.71
Clupea	Atlantic herring	1	135	2.50	24.76	5.30
Citharichthys	Gulf Stream flounder	1	130	2.41	21.95	9.75
Lophius	Monkfish	1	123	2.28	5.96	4.27
Scophthalmus	Windowpane	1	90	1.67	96.33	24.48
Scomber	Atlantic mackerel	1	75	1.39	70.86	6.88
Pollachius	Pollock	1	66	1.22	25.95	5.32
Liparis	Snailfish	2	65	1.21	21.45	11.11
Ulvaria	Radiated shanny	1	57	1.06	26.55	11.82
Lumpenus	Shanny, eelblenny	3	55	1.02	5.85	3.74
Myoxocephalus	Sculpin	3	45	0.83	10.53	6.52
Brosme	Cusk	1	38	0.70	14.67	6.20
Bothus	Eyed flounder	1	37	0.69	3.30	2.82
Notolepis	Barracudina	1	34	0.63	4.80	3.49
Ceratoscopelus	Lanternfish	2	25	0.46	20.75	4.72
Triglops	Moustache sculpin	1	25	0.46	6.56	4.34
Aspidophoroides	Alligatorfish	1	17	0.32	4.93	4.17
Lampanyctus	Lanternfish	3–6	15	0.28	3.21	2.34
Mallotus	Capelin	1	15	0.28	4.94	3.27
Pleuronectes	Winter flounder	1	14	0.26	12.20	9.59
Cryptacanthodes	Wrymouth	1	13	0.24	3.49	3.12
Protomyctophum	Protomyctophum	1	13	0.24	2.49	1.89
Stomias	Dragonfish	1–2	12	0.22	1.93	1.85
Anarhichas	Wolffish	3	10	0.19	5.55	3.81
Myctophum	Lanternfish	2	10	0.19	3.11	2.07
Stichaeus	Arctic shanny	1	10	0.19	562.80	5.72
Symbolophorus	Largescale lanternfish	1	10	0.19	2.63	2.28
Cyclothone	Anglemouth	4	9	0.17	6.37	3.52
Ophichthus	Eels	3	9	0.17	2.78	2.52
Pholis	Rock gunnel	1	9	0.17	11.44	11.85
Howella	Howella	1	7	0.13	4.19	2.04
Maurolicus	Mullers pearlsides	1	7	0.13	3.89	4.72
Anguilla	American eel	1	6	0.11	2.44	2.05
Lobianchia	Lanternfish	2	6	0.11	2.59	2.19
Peprilus	Butterfish	1	6	0.11	15.09	5.33
Echiodon	Chain pearlfish	1	5	0.09	2.27	2.02
Hygophum	Lanternfish	2	5	0.09	1.76	1.72
Scomberesox	Saury	1	5	0.09	4.99	4.70
Reinhardtius	Greenland halibut	1	4	0.07	3.06	2.82
Vinciguerria	Lightfish	3	4	0.07	3.91	1.93
Argentina	Argentine	2	3	0.06	2.12	1.97
Hildebrandia	Conger	?	3	0.06	2.32	1.80
Paraconger	Conger	?	3	0.06	1.70	1.70
Bathylagus	Deepsea smelts	2	2	0.04	1.86	1.86
Chauliodus	Viperfish	2	2	0.04	1.48	1.48

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Table 2 (*concluded*).

Genus	Common name(s)	No. of species	Frequency	% of total count	Mean abundance	Median abundance
Diaphus	Lanternfish	8	2	0.04	3.44	3.44
Gigantactis	Anglerfish	2	2	0.04	3.94	3.94
Icelus	Sculpin	2	2	0.04	4.03	4.03
Nemichthys	Snipe eel	1	2	0.04	1.52	1.52
Paralepis	Barracudina	1	2	0.04	4.45	4.45
Peristedion	Armored searobin	1	2	0.04	2.75	2.75
Pontinus	Scorpionfish	1	2	0.04	2.25	2.25
Scorpaena	Smoothhead scorpionfish	1	2	0.04	1.49	1.49
Syacium	Channel flounder	1	2	0.04	5.76	5.76
Apogon	Cardinalfish	2	1	0.02	2.52	2.52
Argyropelecus	Hatchetfish	5	1	0.02	1.72	1.72
Aristostomias	Loosejaw	4	1	0.02	4.30	4.30
Artediellus	Hookear sculpin	2	1	0.02	5.70	5.70
Bascanichthys	Sand-eel	?	1	0.02	1.89	1.89
Bolinichthys	Lanternfish	1	1	0.02	1.72	1.72
Diplogrammus, *Foetorepus*, and *Paradiplogrammus*	Dragonets	3	1	0.02	0.75	0.75
Gasterosteus	Stickleback	2	1	0.02	6.17	6.17
Gonichthys	Gonichthys	1	1	0.02	6.61	6.61
Gonostoma	Longtooth anglemouth	1	1	0.02	1.95	1.95
Gymnothorax	Green moray	1	1	0.02	1.21	1.21
Hippocampus	Lined seahorse	1	1	0.02	4.10	4.10
Hoplunnis	Hoplunnis	?	1	0.02	3.20	3.20
Leptagonus	Atlantic poacher	1	1	0.02	15.65	15.65
Myrophis	Worm eel	2	1	0.02	1.86	1.86
Nessorhamphus	Duckbill oceanic eel	1	1	0.02	1.71	1.71
Nezumia	Marlin-spike	1	1	0.02	1.28	1.28
Notolychnus	Notolychnus	1	1	0.02	1.95	1.95
Notoscopelus	Notoscopelus	1	1	0.02	1.94	1.94
Phycis	Hake	?	1	0.02	4.28	4.28
Prionotus	Northern searobin	1	1	0.02	5.16	5.16
Psenes	Driftfish	2	1	0.02	1.87	1.87
Svetovidovia	Svetovidovia	?	1	0.02	5.16	5.16
Synodus	Red lizardfish	1	1	0.02	4.30	4.30
Uroconger	Threadtail conger	1	1	0.02	1.44	1.44
Valenciennellus	Valenciennellus	1	1	0.02	1.72	1.72

Note: Genera are ordered by frequency of occurrence.

0.001) (Fig. 3*b*). Shannon's H was not related to log abundance in winter and in summer–fall and was negatively correlated in spring–summer ($r = -0.12$, $p = 0.003$, $n = 593$). A preliminary interpretation would be that logGR and log abundance were correlated because they were positively related to area sampled or to the number of individuals sampled. It is common to observe that the number of taxa encountered increases with sample size up to a given level, after which it stays constant. Sample size is one of various causes of a "species–area" curve (MacArthur 1965; Magurran 1988). In our study, the volume towed (sample size) was not significantly related to the number of either genera or individuals, holding sampling depth constant. Because samples were standardized, uniform, and small relative to the area of the shelf, we would not expect log GR or H to be related to sample size.

In areas of high GR, abundance is high, whereas in areas of high H, abundance is low (Fig. 4). Given that GR and H are positively related, how is it that they show different patterns with abundance? The difference is that GR is simply a measure of the number of genera, whereas H is a measure of the number of genera while accounting for varying abundances. Although the number of genera can result in an increase in abundance, a corresponding decrease in H reflects that an increase in abundance is increasingly dominated by fewer genera. This explanation is borne out by the shape of frequency of abundance distributions. Skewness was lowest in winter (skewness = 1.3, SE = 0.12), higher in summer–fall (skewness = 0.65, SE = 0.069), and highest in spring–summer (skewness = 0.4, SE = 0.097).

We analysed the spatial distribution of diversity (GR and H) and abundance. Data were plotted into 0.031 decimal degree grids for the purposes of contouring. In general, the distribution of empty sets was greater in winter (Figs. 4*a*–4*c*), reflecting both a lower diversity and a more limited distribution. GR, H, and abundance were high in the Western – Sable

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Fig. 2. (*a*) Monthly log GR ($\log_{10}$(number of genera + 1)·1000 m^{-3}), (*b*) H, and (*c*) log abundance ($\log_{10}$(number of individuals + 1)·1000 m^{-3}). Boxes represent interquartile range (50% of data), the horizontal line within the box represents the median, circles represent 1.5–3 box lengths from the edge of the box, and asterisks represent >3 box lengths from the outer edge of the box.

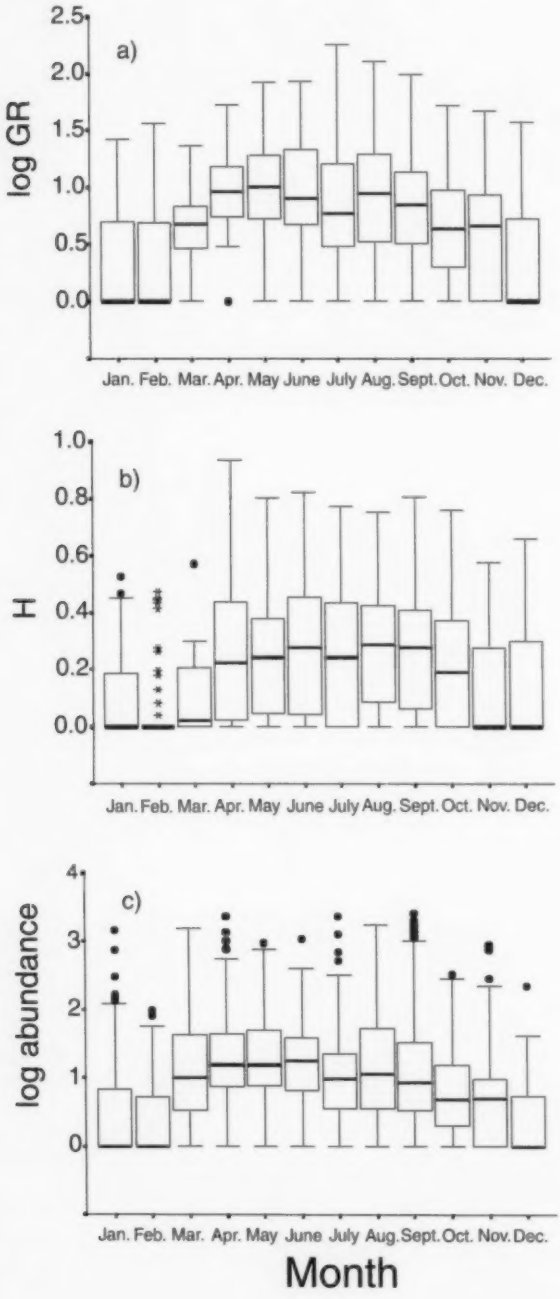

Fig. 3. Relationships between (*a*) log GR ($\log_{10}$(number of genera + 1)·1000 m^{-3}) and *H*, (*b*) log GR and log abundance ($\log_{10}$(number of individuals + 1)·1000 m^{-3}), and (*c*) *H* and log abundance. In Fig. 3*c*, seasons are represented by plus signs (winter), open circles (spring–summer), and solid circles (summer–fall).

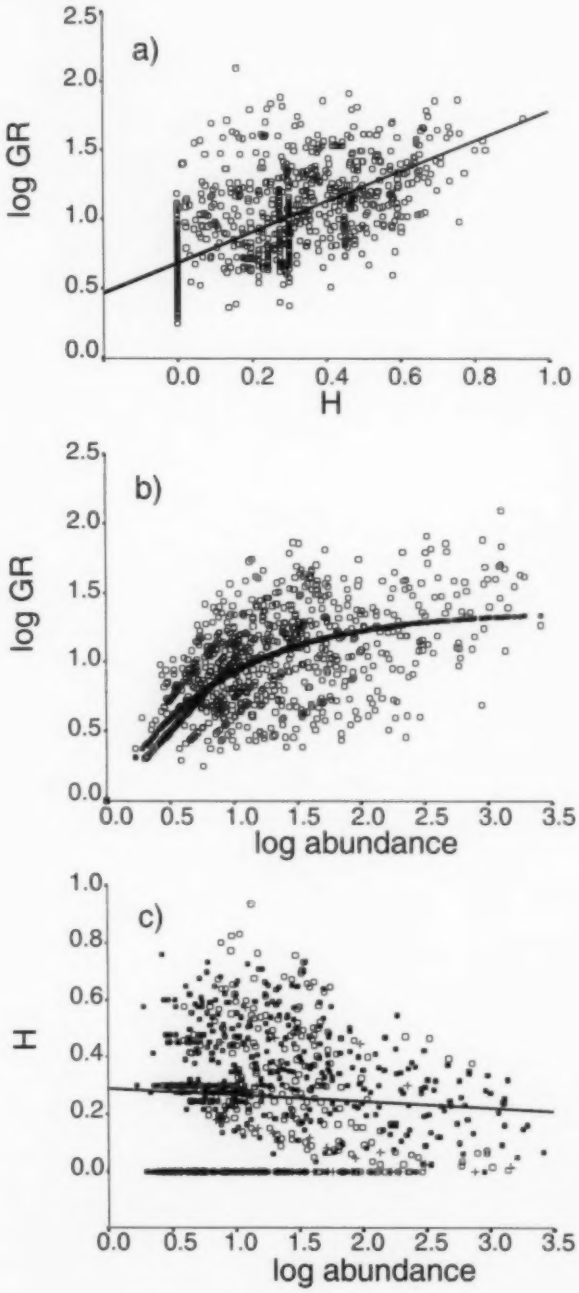

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Table 3. Cluster groups as recognized by hierarchical agglomerative cluster analysis.

Cluster	Genus	Egg type	Cluster	Genus	Egg type
1: shallow, cool	*Leptagonus*	ProbD		*Myoxocephalus*	D
	Hippoglossoides	P		*Stichaeus*	D
	Clupea	D		*Pholis*	D
	Melanogrammus	P		*Pollachius*	P
	Liparis	D		*Anarhichas*	D
	Gadus	P		*Cryptacanthodes*	ProbD
	Pleuronectes (winter flounder)	D		*Lumpenus*	D
	Phycis	P		*Artediellus*	D
2: shallow, warmest	*Apogon*	D		*Anguilla*	Unknown
	Hoplunnis	Unknown		*Reinhardtius*	P
	Ophichthus	Unknown	6: middepth, warmer	*Argentina*	P
	Psenes	P		*Maurolicus*	P
	Scomberesox	P		*Bolinichthys*	ProbP
	Aristostomias	Unknown		*Protomyctophum*	Unknown
	Synodus	P		*Echiodon*	P
	Citharichthys	Unknown		*Paraconger*	Unknown
	Glyptocephalus	P		*Vinciguerria*	P
	Prionotus	P		*Uroconger*	Unknown
	Svetovidovia	ProbP	7: middepth, cool/warm	*Benthosema*	Unknown
	Urophycis	P		*Chauliodus*	O
	Pleuronectes (yellowtail flounder)	P		*Nemichthys*	Unknown
	Merluccius	P		*Paralepis*	Unknown
	Scophthalmus	P	8: deep, warmest	*Ceratoscopelus*	ProbP
	Gasterosteus	D		*Scorpaena*	P
	Peprilus	P		*Myctophum*	Unknown
	Scomber	P		*Symbolophorus*	Unknown
	Bascanichthys	Unknown		*Gigantactis*	Unknown
	Enchelyopus	P		*Howella*	P
	Lophius	P		*Lampanyctus*	Unknown
	Tautogolabrus	P	9: deep, warm	*Myrophis*	Unknown
	Syacium	P		*Nezumia*	ProbP
3: deep, cold	*Argyropelecus*	P		*Notolepis*	Unknown
	Bathylagus	P		*Stomias*	O
4: deep, warmest	*Diplogrammus*, *Foetorepus*, and *Paradiplogrammus*	P	10: middepth, warmest	*Bothus*	ProbP
	Hildebrandia	Unknown		*Pontinus*	Unknown
	Lobianchia	ProbP		*Diaphus*	ProbP
	Hygophum	ProbP		*Cyclothone*	Unknown
	Gymnothorax	Unknown		*Peristedion*	P
	Notoscopelus	Unknown	11: shallow, warm	*Brosme*	P
	Valenciennellus	P		*Hippocampus*	ProbO
5: shallow, cold	*Ammodytes*	D		*Ulvaria*	D
	Triglops	ProbD		*Mallotus*	D
	Aspidophoroides	ProbD		*Sebastes*	O
	Icelus	ProbD			

Note: Egg type: P, pelagic; D, demersal; O, oviviparous (ProbO, probably oviviparous; ProbD is probably demersal and ProbP is probably pelagic, as inferred by Lou Van Guelpen (Atlantic Reference Centre, St. Andrew's by the Sea, NB EJB 2L7, Canada, personal communication: arc@mar.dfo-mpo.gc.ca)). Range of depths observed: shallow (<200 m), middepth (200–600 m), and deep (>600 m) water. Within depth ranges, assemblages occurred in a range of temperatures roughly categorized as cold (<6°C), cool (>5°C and <10°C), warm (>9°C and <12.5°C), warmer (>12.4°C and <15°C), and warmest (>15°C).

Island banks area compared with the rest of the shelf (Figs. 4*a*–4*c*). During spring–summer, GR and *H* were both higher on LaHave, Emerald, Western – Sable Island, and Georges banks (Figs. 4*d* and 4*e*). GR was also high on Browns Bank and Banquereau (Fig. 4*d*). Abundance was relatively higher in the Middle, Canso, and Misaine banks and Banquereau areas and to a lesser extent in Browns and Sable Island bank areas (Fig. 4*f*). During summer–fall, GR and abundance remained high on Western – Sable Island banks, whereas *H* did not (Figs. 4*g*–4*i*).

Larval fish assemblages

The five most frequently occurring genera during winter were *Ammodytes* (26.8%), *Clupea* (19.0%), *Pollachius* (12.8%), *Gadus* (10.6%), and *Lumpenus* (5.6%). During spring–summer, the most frequently encountered genera

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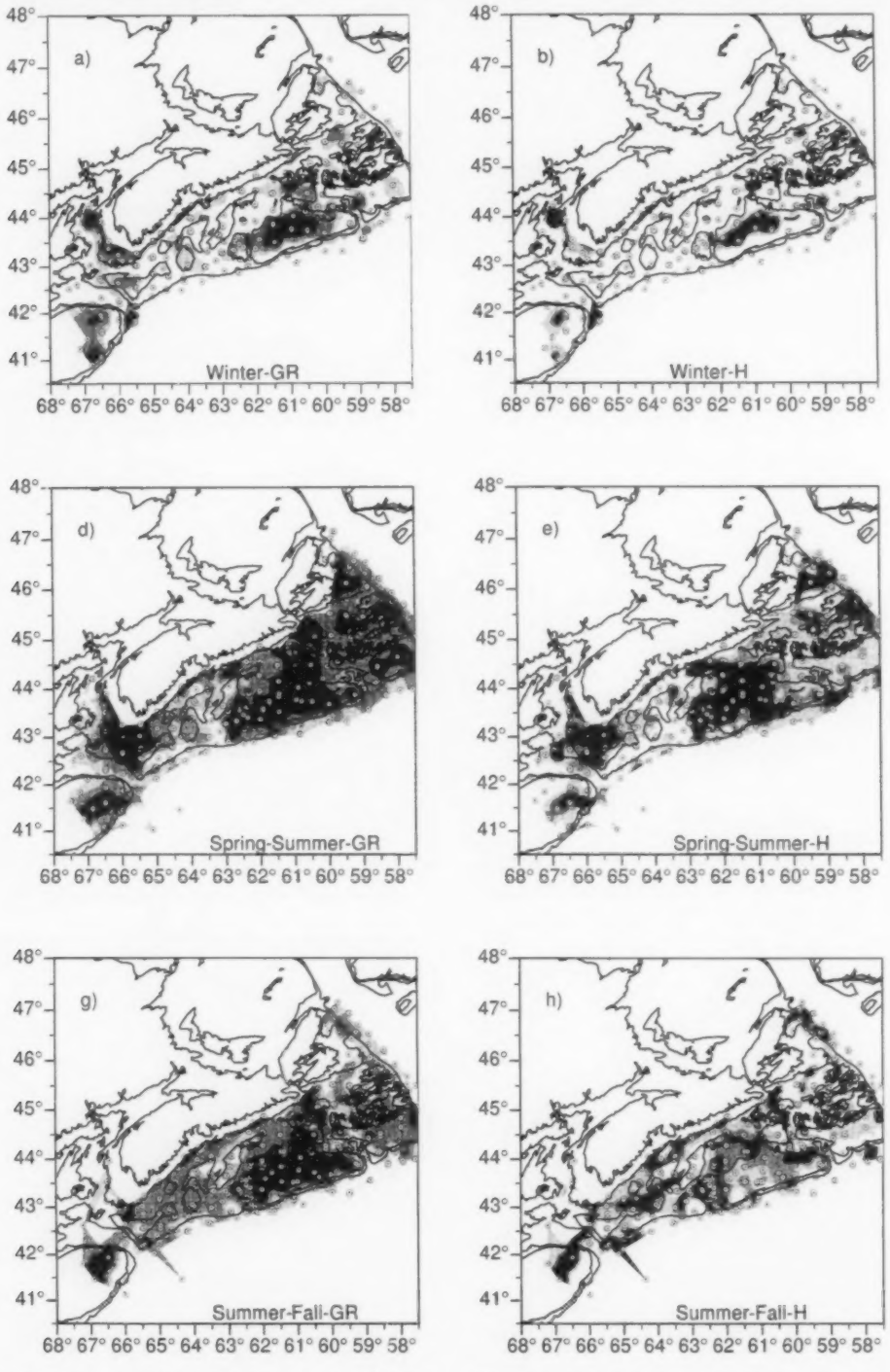
Fig. 4. Seasonal distribution of (*a*, *d*, *g*) log GR, (*b*, *e*, *h*) *H*, and (*c*, *f*, *i*) log abundance: (*a–c*) winter, (*d–f*) spring–summer, and (*g–i*) summer–fall. Contour shades ranging from light to dark represent the 0, 25, 50, 75, and 100 percentiles for each metric.



were *Sebastes* (18.6%), *Ammodytes* (16.6%), *Gadus* (8.8%), *Hippoglossoides* (8.2%), and *Melanogrammus* (7.6%). During summer–fall, *Merluccius* (21.9%), *Urophycis* (16.1%), *Glyptocephalus* (11.3%), *Enchelyopus* (7.6%), and *Sebastes* (7.2%) were the most frequently encountered genera.

Associations among taxa based on cluster analysis re-

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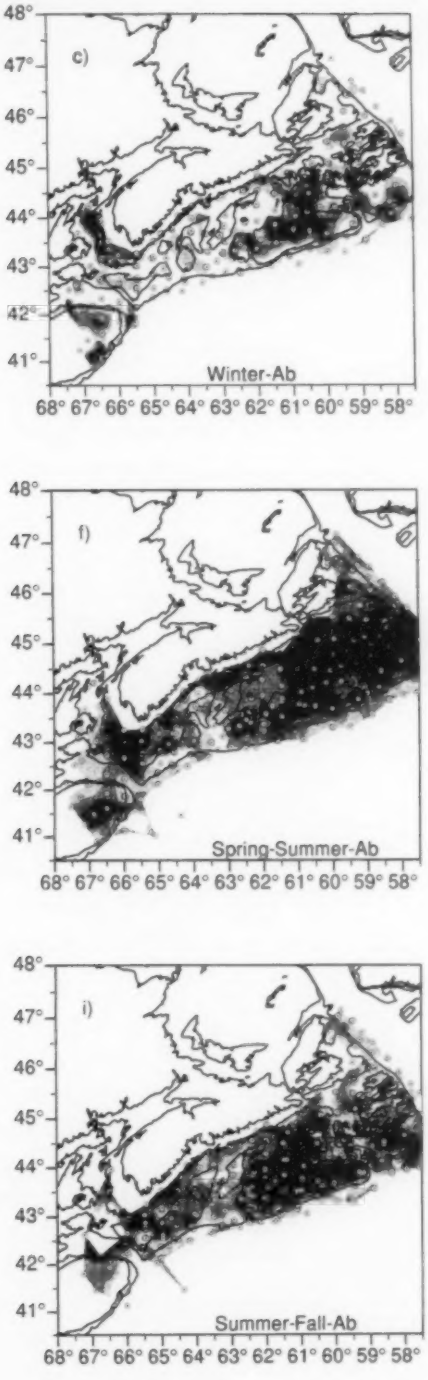
Fig. 4 (*concluded*).



vealed 11 assemblages (Table 3; Figs. 5 and 6). Assemblages occurred over a range of depths roughly categorized as shallow (<200 m), mid-depth (200–600 m), and deep (>600 m) water. Within these depth ranges, assemblages occurred in a range of temperatures roughly categorized as cold (<6°C), cool (>5°C and <10°C), warm (>9°C and <12.5°C), warmer (>12.4°C and <15°C), and warmest (>15°C). These loosely defined temperature–depth categories have been used to describe the habitat type of each of the 11 assemblages (Table 3). To some extent, the assemblages aligned along the temperature axis (Fig. 6) represent seasonally occurring assemblages. For example, cluster 5 represents larvae occurring in cold water. Larvae that originated from demersal eggs dominated cluster 5 (Table 3) and their peak abundance occurred from November to June (Fig. 7*a*). Cluster 1 represents larvae that occurred in cool water. The peak larval abundance for cluster 1 occurred from April to June and October to December; the latter peak reflects the influence of the fall-spawning *Clupea* and *Gadus* components (Fig. 7*b*). Cluster 11 occurred in warmer water; peak larval abundance occurred from June to July (Fig. 7*c*). Cluster 2 represents larvae that occurred in warm water collected over shallow depths, the hakes and flounders. Abundance peaked in July (Fig. 7*d*). Clusters 4, 9, 8, 6, and 10 occurred in relatively warmer, deeper water and represent larvae of mesopelagic and deepwater fishes. Clusters 7 and 3 occurred in cooler water over greater depths and represent bathypelagic or deepwater fishes.

Discussion

Diversity, abundance, and prey

To examine the temporal correspondence between food availability and diversity, we used published information on zooplankton collected during the SSIP developed by Tremblay and Roff (1983), O'Boyle et al. (1984), Sameoto and Herman (1992), and Brander and Hurley (1992). In general, the zooplankton community composition can be related to hydrographic variables and varies spatially and temporally from inshore to offshore (Tremblay and Roff 1983) and by latitude (Sameoto and Herman 1992). During the SSIP 1979–1980 surveys, the zooplankton biomass peaked in May (O'Boyle et al. 1984). As reported by Sameoto and Herman (1992), the average concentration of *Calanus finmarchicus*, *C. glacialis*, and *C. hyperboreus* peaked from May to June and there was suggestion of a lesser peak in September. In our study, there was no obvious "peak" in larval diversity. Rather, diversity was relatively higher over a prolonged period (April to September–October). Because we might have obscured smaller-scale patterns at the shelf-scale level of analysis, we examined temporal patterns in diversity separately for the eastern and western Scotian Shelf (as defined by longitude 63.49°). There were no apparent peaks in diversity within regions.

In general, the Scotian Shelf supports a changing array of larval fish throughout the year. The taxon composition is dynamic, but the level of diversity is fairly constant through spring–summer and early fall. There is a seasonal component to taxon composition and this is apparent in how the cluster groups are aligned on a surface temperature axis, as well as the seasonal progression of abundance. Interestingly, the peak abundance of members of cluster 1 occurs in spring and fall. This cluster contains taxa, such as *Gadus* and *Melanogrammus*, that conform to the conventional notion of a co-occurrence between larval fish and calanoid copepods.

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Although Brander and Hurley (1992) and Sherman et al. (1984) suggested that various genera may be taking advantage of a spring and fall bloom, Leggett and Deblois (1994) could report only limited evidence to support the match–mismatch hypothesis. Further, Mousseau et al. (1998) suggested that, in general, larvae are not dependent on peak zooplankton blooms. They showed that larval fish production on Sable Island Bank can be partially supported by the microbial food web and may not be entirely dependent on spring and fall blooms. Both calanoid and cyclopoid copepods can prey on members of the microbial food web. A diversity of food webs may exist and different genera may use different food resources, and this may account for the fairly constant levels of diversity and the variation in larval abundance observed in this study throughout spring–summer and early fall. Our results suggest that there is substantial ecological latitude to support a variety of larval fish types throughout most of the year, and they support the assertion of Mousseau et al. (1998) that the match–mismatch hypothesis has only limited application. A limitation of our analysis is that we combined data from different years to create a seasonal cycle. Indeed, a lack of correspondence between larval diversity and zooplankton abundance could result from our methodology or because we are comparing larval and zooplankton abundance at too large a scale. We favor the interpretation that the stability of GR and H through spring–summer and early fall reflects that calanoid copepods do not have to be the direct or sole food source for the majority of larval fish. This is supported by the observation that peak abundances of various assemblages vary throughout the season and that the peak abundance of cluster 1 occurs during spring and fall, as would be predicted given the spawning characteristics of the cluster members (e.g., *Gadus*, *Melanogrammus*, *Clupea*).

In several plant, terrestrial, and deep-ocean studies, diversity exhibits a dome-shaped relationship with productivity (reviewed in Rosenzweig and Abramsky 1993). Assuming that abundance is an indirect measure of productivity, our results support that observation; GR increased with abundance, reaching an asymptote at higher levels of abundance. Regions with high abundance have sufficient resources to support a higher richness, but diversity is dominated by fewer genera as abundance increases. Interestingly, the spatial pattern of zooplankton collected during the SSIP accords well with the spatial pattern of larval fish diversity that we observed. O'Boyle et al. (1984) described zooplankton biomass to be highly concentrated on Emerald and Western banks and the eastern end of Sable Island Bank and highest over Misaine Bank. This would support the notion that a sufficient resource base characterizes areas of relatively high richness and abundance.

Diversity, abundance, and oceanographic circulation

Does the surface circulation correspond to patterns of diversity and abundance? The most striking feature was that a partial gyre encircling the Western – Sable Island banks area in winter and summer (Fig. 8) (C. Hannah, Department of Fisheries and Oceans, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada, personal communication; Cong et al. 1996) corresponded to high levels of diversity and abundance in that area. Gyres may promote retention and survival in gadoid larvae (Werner et al. 1996; Shackell et al. 1999). It is possible that gyres may do so in other taxa, resulting in higher richness and (or) abundance associated with gyres. However, higher diversity is not exclusively observed in areas that promote retention, nor is it consistently higher in areas where there are gyres. The very general correspondence between circulation and diversity may reflect the scale of our study. Certainly, a drawback of many studies of larval assemblages and hydrography is the common attempt to relate average, or climatological, conditions (Cowen et al. 1993). We have compared the climatological circulation with larval diversity as determined from the SSIP years. A smaller-scale study may have revealed larval associations with fronts and more highly resolved gyres. The alternative is that despite the governing hypothesis that retention favors survival, many genera collected herein have an alternative strategy: maximum dispersal of larvae, instead of maximum retention, is more favorable to survival.

We observed high levels of GR and abundance on Western – Sable Island banks. What are the mechanisms that support richness and abundance in that area? A species–area relationship describes the common observation that the number of species increases as area increases (MacArthur 1965). There are various causes of a species–area relationship including an increase in within- and (or) between-habitat diversity concomitant with an area increase until habitats are saturated. Certainly, the Western – Sable Island banks region is larger than other banks. It could also be considered a transitional zone between two or three distinct ecosystems, the cooler eastern Scotian Shelf, the warmer western Scotian Shelf, and the shelf-slope region. *Merluccius* and *Urophycis*, for example, occur primarily on Western – Sable Island banks and west, whereas *Pleuronectes* (yellowtail flounder) occurs primarily on Western – Sable Island banks and east. Western – Sable Island banks may contain more heterogeneous habitat and that may sustain a greater variety of fish. In addition, as noted above, zooplankton biomass on Western – Sable Island banks has been observed to be relatively high (O'Boyle et al. 1984). If this feature were consistent over time, it may itself be governed by the circulation and support a relatively greater number of species. We propose that GR is relatively high in the Western – Sable Island banks area because it is a larger bank with relatively higher habitat heterogeneity and a large partial gyre that serves as a retentive mechanism and to concentrate both larval fish and their prey.

Diversity and conservation strategies

We chose a shelf-scale level of analysis to describe larval diversity in part because there is little shelf-wide information on larval fish assemblages and because our results may have application to monitoring programs, to the delineation of shelf-scale ecosystems, and to the identification of potential protected areas.

Diversity is often used as one of a suite of criteria to describe ecosystems or to identify potential protected areas. There is considerable debate as to the value of diversity and its role as an ecological process. In the marine environment, there is some information on patterns of diversity (Ormond et al. 1997), yet there exists little empirical information on the relationship between marine diversity, stability, and (or)

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Fig. 5. Dendrogram of hierarchical relationships among larval fish genera, as determined by hierarchical agglomerative cluster analysis. Common symbols denote members of the same cluster. Di, Fo, and Pa, *Diplogrammus*, *Foetorepus*, and *Paradiplogrammus*.

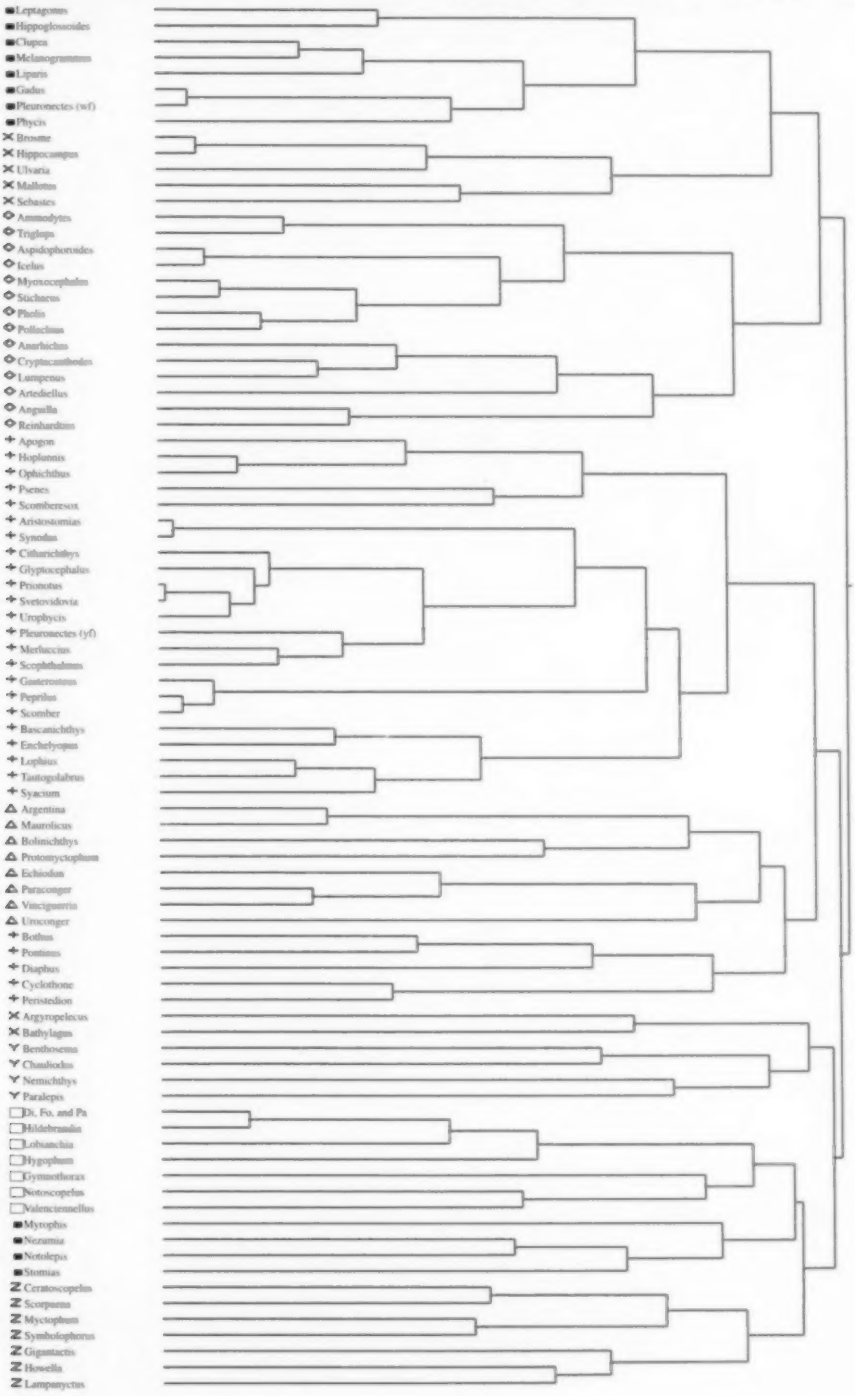

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Fig. 6. Cluster groups as per surface temperature and bottom depth. Symbols correspond to closest cluster labels (1–11), which correspond to cluster groups in Table 3. Solid circles, cluster 1; open triangles, cluster 2; open diamonds, cluster 3; open squares, cluster 4; solid triangles, cluster 5; solid squares, cluster 6; open circles, cluster 7; solid diamonds, cluster 8; ×'s, cluster 9; asterisks, cluster 10; plus signs, cluster 11.

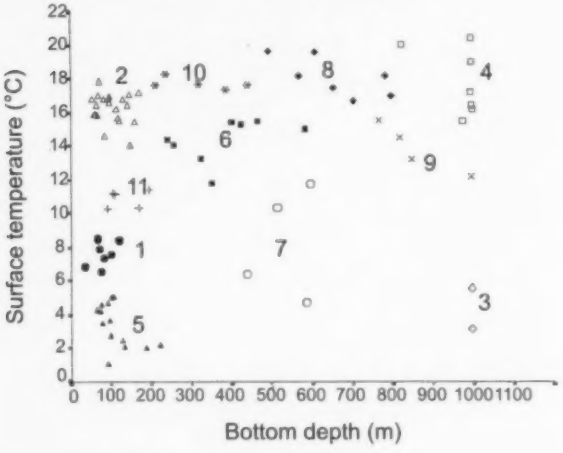
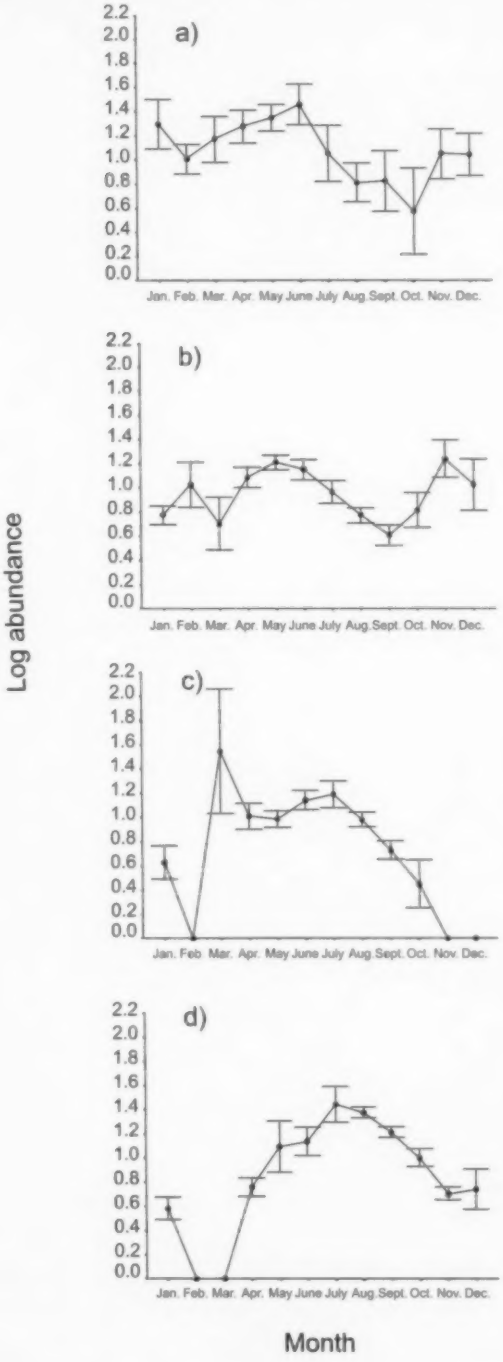


Fig. 7. Monthly mean and SEM of log abundance of four clusters, (*a*) 5, (*b*) 1, (*c*) 11, and (*d*) 2, showing seasonal progression of abundance.



resilience (Allison et al. 1996). Further to that is the observation that the pattern of diversity of one group does not necessarily coincide spatially or temporally with that of another (Nott and Pimm 1997). Where does our study fit in the rapidly evolving field of conservation?

Larval assemblages are dynamic and may have changed since the SSIP, particularly on the eastern Scotian Shelf where Atlantic cod have collapsed and the fish community composition has changed (Frank et al. 1996). Biodiversity in general is changeable and is a function of biophysical processes. Because of potential change, conservation measures ideally would be based on those processes that govern its level and composition. Because processes are difficult to identify in the short term, the use of landscape- or habitat-level features as descriptors of community diversity has been proposed (Angermeier and Winston 1999; Ward et al. 1999). We have identified seasons and areas that were favorable to larval finfish survival on the Scotian Shelf during the SSIP. A further question is which habitat features/descriptors and what scales are appropriate for conservation objectives.

We showed that (*i*) diversity and abundance were relatively higher from April to September–October and that (*ii*) the level of diversity from April to September–October was stable but the taxon composition changed. From this, we inferred that the majority of taxa occurring on the Scotian Shelf were not dependent on a peak spring or fall bloom of calanoid copepods. Instead, as Mousseau et al. (1998) have proposed, there is a diversity of food webs supporting larval fish production. We also showed that (*iii*) GR was positively related to abundance but that the relationship asymptotes at higher levels of abundance and that (*iv*) *H*, which measures both diversity and evenness, was either unrelated or negatively related to abundance. Thus, diversity increases with abundance but the composition is dominated by relatively fewer genera at higher levels of abundance. We also showed that (*v*) various banks were diverse and (or) productive areas, especially Western – Sable Island banks, and that the latter was associated with a large gyre that could act as a retentive

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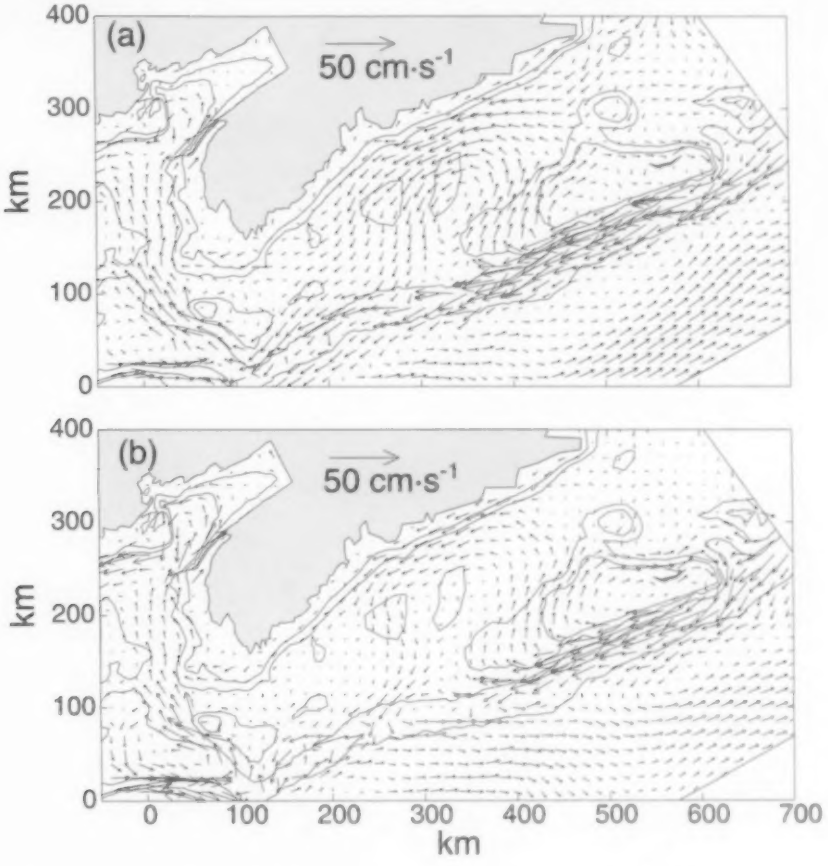
Fig. 8. Mean velocities, averaged between 20 and 50 m, during (*a*) January–February and (*b*) July–August as estimated using a physical circulation model (reprinted with permission from the Journal of Physical Oceanography (Hannah et al. 2000)).



mechanism and could concentrate both larval fish and their prey. In this initial contribution, we did not address those smaller-scale associations, e.g., whether larvae were concentrated in fronts coincident with their food (see McLaren et al. 1997). Smaller-scale research should reveal whether circulation in high-diversity areas enhances larval survival and whether the temporal and spatial variability in larval finfish diversity reflects an abundant and (or) diverse food supply.

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Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland

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Abstract: We provide evidence that variation in location and timing of spawning of Atlantic cod, *Gadus morhua*, has a major impact on occurrence, distribution, and development of eggs and larvae in a large embayment on the south coast of Newfoundland. Atlantic cod egg densities, measured with a series of ichthyoplankton surveys in Placentia Bay during the spawning and postspawning seasons of 1997 and 1998, were highest during the early spring of both years and decreased through the spring and summer. Egg development stages and larval size suggest that eggs and larvae were released from spawning locations within the bay and developed as they were transported in cyclonic flow from the southeast and around the bay towards the southwest, where late-stage eggs and larvae were found to be most abundant. Although egg densities were generally lower in 1998, late-season egg production during the summer of 1998 was elevated in relation to 1997, providing a possible explanation for the observation that larval total densities were more than an order of magnitude higher in 1998 than in 1997. In terms of larval production, late spawners may be particularly important to successful egg hatching in the coastal waters of Newfoundland.

Résumé : Nous montrons que la variation dans le lieu et le moment de la ponte chez la morue, *Gadus morhua*, a un impact majeur sur la présence, la distribution et le développement des oeufs et des larves dans une grande baie de la côte sud de Terre-Neuve. Les densités des oeufs de morue, mesurées à partir des données d'échantillonnages de l'ichthyoplancton réalisés dans la baie Placentia durant et après la ponte en 1997 et 1998, étaient plus élevées au début des deux printemps et diminuaient ensuite durant le reste du printemps et l'été. Les stades de développement des oeufs et la taille des larves laissent penser que les oeufs et les larves ont été entraînés hors des lieux de ponte de la baie et se sont développés durant leur transport dans un flux cyclonique depuis le sud-est jusqu'au sud-ouest de la baie, où les oeufs bien développés et les larves étaient les plus abondants. Bien que les densités des oeufs étaient généralement faibles en 1998, la production d'oeufs en fin de saison durant l'été de 1998 était élevée par rapport à celle de 1997, ce qui pourrait expliquer que les densités totales de larves en 1998 étaient de plus d'un ordre de grandeur supérieures à celles observées en 1997. Les poissons qui pondent tardivement pourraient contribuer de façon particulièrement importante à la production de larves dans les eaux côtières de Terre-Neuve.

[Traduit par la Rédaction]

Introduction

The placement of reproductive propagules into an environment where they are likely to hatch and settle into an appropriate habitat is thought to play a large role in ensuring successful recruitment in marine fish (Harden Jones 1969; Sinclair 1988). In Atlantic cod, *Gadus morhua*, there is increasing evidence that spawning occurs in multiple coastal areas of Newfoundland (Smedbol et al. 1998; Lawson and Rose 2000). Compared with offshore habitats, coastal spawning may result in higher survival because of decreased predation and higher growth rates resulting from higher prey densities (Frank and Leggett 1982; Taggart and Leggett 1987; Pepin et al. 1995). The importance of inshore spawners for Atlantic cod off Newfoundland has been compounded in recent years, as a larger portion of the spawner biomass is now found in the bays along the coast (Anonymous 1998; Lawson and Rose 2000) and the offshore regions show no signs of recovery (Shelton and Healey 1999). In coastal Newfoundland, a recent study observed no recruitment signal from an unusually large inshore spawning aggregation (Smedbol et al. 1998), raising the question of whether spawning patterns have a major effect on recruitment success. Whether coastal spawning is likely to confer any eventual habitat advantage for juveniles depends on successful hatching near the coastal habitat, which in turn depends on timing and location of spawning in relation to water temperature and surface currents.

The idea that Atlantic cod egg (defined here as all pelagic,

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prehatch stages) and larval (pelagic, posthatch individuals) survival and subsequent recruitment are linked to variation in retention and spawning has been proposed by deYoung and Rose (1993) as the "right site" hypothesis. These authors hypothesized that during cold years, Atlantic cod on the Newfoundland shelf shifted their spawning distribution to the south where egg retention was low, resulting in years of poor recruitment. This hypothesis has been supported by the co-occurrence of cold conditions, southerly spawning distributions, and poor recruitment during the early 1990s (Rose et al. 2000). In coastal areas, currents may be less influenced by the Labrador Current and more by local processes, such as wind forcing (deYoung et al. 1993). Transport of ichthyoplankton in Conception Bay, Newfoundland, was examined by Pepin et al. (1995) who concluded that wind stress contributed to transport and changes in larval fish abundance.

Atlantic cod spawn over a protracted period from March to September on the coast of Newfoundland, thus greatly increasing the potential range of conditions during which egg and larval development occurs (Myers et al. 1993; Hutchings and Myers 1994). Although it is known that density-dependent factors vary seasonally (Cushing 1990), environmental factors such as water temperature and wind stress, which influence egg and larval distributions and vital rates, may also display distinct seasonal cycles off the coast of Newfoundland (Petrie et al. 1991). Thus, the effect of environmental conditions in a given year and a comprehensive evaluation of environmental regulation of egg and larval abundance require sampling throughout the spawning peak as well as detailed knowledge of spatial and temporal variation in spawning biology.

We hypothesize that spatial and temporal pattern and survival of planktonic eggs and larvae are dictated by adult distribution and spawning characteristics, local and nonlocal spawning (Pepin and Helbig 1997; Leising and Franks 1999), environmental conditions (McGurk 1986; Laprise and Pepin 1995), and transport processes (Pepin et al. 1995). Moreover, we speculate that interactions between these factors will contribute to the spatial and temporal pattern of eggs and larvae, which will ultimately control the growth and survival of Atlantic cod larvae. Although there have been detailed studies on each of these factors in relation to Atlantic cod, few have attempted to link them within a single study. Nonetheless, the importance of understanding the individual factors contributing to patterns in the spatial and temporal distribution of eggs and larvae remains paramount, given that these factors can dramatically influence recruitment variability in Atlantic cod (Sinclair 1988; deYoung and Rose 1993).

We have focused our study on Placentia Bay, Newfoundland, because it is one of the few areas in Atlantic Canada where spawner biomass and, therefore, egg and larval supply are considered to be reasonably healthy at this time. The absence of sea ice also allowed sampling throughout the spawning period. The objective of this study was to determine how spatial and temporal distribution and development of Atlantic cod eggs and larvae in Placentia Bay are related to adult spawning location and timing and to environmental conditions (circulation, temperature, salinity, and chlorophyll *a*). We then consider how year-to-year variation in these factors may ultimately affect the success of larval settlement in coastal habitats. For this study, we were therefore interested in determining whether Atlantic cod spawning in coastal environments resulted in retention where higher availability of food resources and juvenile habitat would result in increased larval survival, thus supporting the right site hypothesis.

Methods

Placentia Bay is a large embayment on the southeast coast of Newfoundland (Fig. 1), measuring ~130 km along a longitudinal axis that runs ~25° east of north and ~100 km wide at the open southern mouth of the bay. Although scattered banks contribute to considerable variability in bottom depth, many areas of the bay are hundreds of metres deep, and the sides of the bay are quite steep. Elongate islands subdivide the northern portion of the bay into three channels, and anecdotal evidence suggests that currents flow into the bay along the eastern side and out along the western side.

Coastal circulation and wind data

In order to describe circulation patterns in Placentia Bay, we used several approaches. First, 500 grapefruit were released as passive drifters from each of two sites near Bar Haven and Perch Rock (see Fig. 1) in June of 1997; both of these sites had been identified from acoustic surveys as major spawning locations in the bay for that year (Lawson and Rose 2000). Grapefruit were individually numbered by burning to allow linkage between release and recovery points. A brief survey of the outer bay the following day failed to locate any of the drifters, and the numerous local fishers working in the area were asked to collect and report any drifters that they encountered. All of the recovered drifters were reported by fishers within 2 weeks of release. Further investigations of the coastal circulation were made in the spring of 1998 using a pair of s4 current meters (B. deYoung, Department of Physics and Physical Oceanography, Memorial University of Newfoundland, unpublished data) moored at a depth of 10 m on opposite sides of the bay (47°14.1′N, 54°37.1′W and 47°07.3′N, 54°10.5′W) from April to mid-June.

Wind data for 1997 and 1998, obtained from Environment Canada, Meteoroligical Service of Canada, was recorded at a station in Argentia (Fig. 1) on the eastern side of the bay (recorded on a tower 10 m high). The data were rotated 25° to align with the axis of the bay, converted to wind stress (Large and Pond 1981), and filtered using an eighth-order Butterworth lowpass filter. The current meter data were similarly rotated and filtered.

Environmental and ichthyoplankton sampling

During the spring and summer of 1997 and 1998, Placentia Bay was surveyed for environmental data along a set survey grid of six parallel transects (see Fig. 1) that ran perpendicular to the long axis of the bay. Transects were 8 nautical miles apart and stations were 4 nautical miles apart along each transect. Vertical CTD (conductivity–temperature–depth sensor) casts for salinity and temperature were collected at each station using either a Seabird 19 (May and June 1997) or a Seabird 25 (all other cruises). Mixed layer temperatures and salinities were determined by averaging the upper 40 m from each profile. For 1998 cruises except April, concentrations of chlorophyll *a* and phaeopigments were measured from triplicate 100-mL samples collected with a 5-L Niskin bottle at 5 m depth and filtered through a Whatman GF/F filter at sea. In the laboratory, samples were extracted in darkness for 24 h in 90% acetone at –20°C and pre- and post-acidification absorbance values (Parsons et al. 1985) were measured with a Turner Designs fluorometer (model AU-10).

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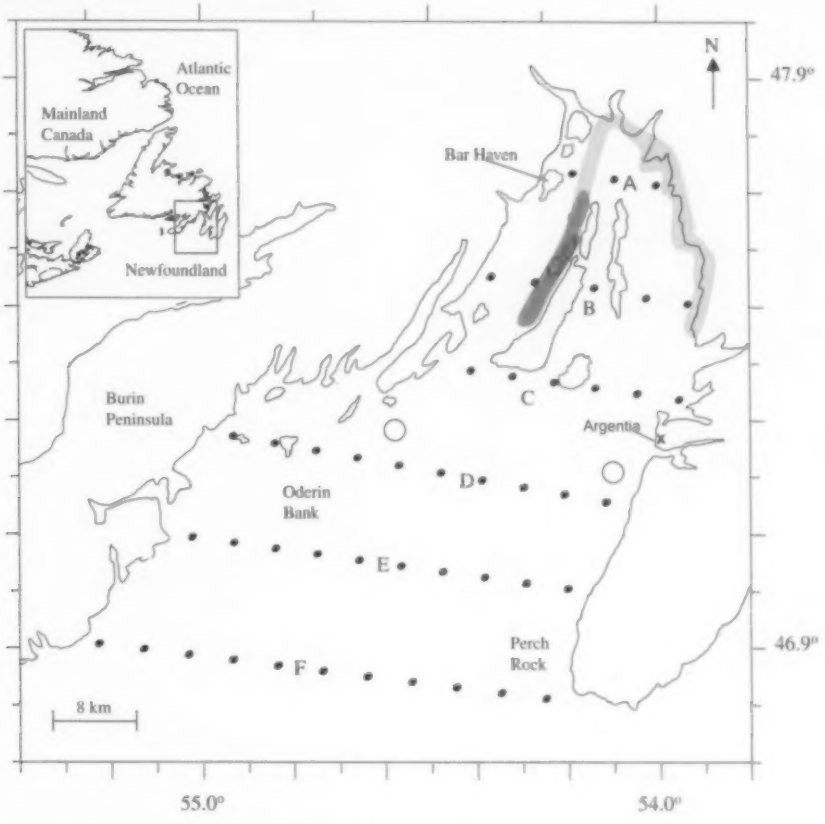
Fig. 1. Chart of Placentia Bay survey transects (A–F) and stations. The inset shows the position of the bay in relation to Newfoundland. Open circles represent s4 current meter mooring locations, and shaded regions show recovery locations of drifters released from Perch Rock (light shading) and Bar Haven (dark shading).



Ichthyoplankton samples were collected concurrently with oceanographic samples at each station. Survey gear consisted of a 2 × 2 m Tucker trawl with decreasing mesh sizes of 1000, 570, and 333 µm. Two General Oceanic flowmeters were placed at the mouth to allow estimates of flow volumes. Double oblique tows to a maximum depth of 40 m and of 15 min duration were collected at a towing speed of ~2 knots. This depth range was chosen because several studies have shown that >95% of the ichthyoplankton in coastal Newfoundland are found in the upper 40 m (Frank and Leggett 1982; deYoung et al. 1993; Laprise and Pepin 1995). Detailed comparisons of Tucker trawl versus bongo samplers (Pepin and Shears 1997) suggest that the Tucker trawl is a more effective sampler because it resulted in higher estimates of species diversity and lower variability, although the Tucker trawl may slightly underestimate larvae under 5 mm. Thus, smaller larvae may be underestimated in our study, but this bias is consistent for all times and locations.

Ichthyoplankton samples were preserved in 4% buffered formalin in seawater. For sample processing, Atlantic cod egg and larval abundances exceeding 300 individuals were subsampled using a Motoda splitter (except April and June 1998, which were processed by the Atlantic Reference Centre and subsampled using the beaker technique of van Guelpen et al. 1982). All eggs and larvae were identified to the lowest taxonomic level possible and measured to the nearest 0.1 and 0.5 mm, respectively. All eggs were grouped into the four development stages presented by Markle and Frost (1985).

Data analysis

Ichthyoplankton abundances were corrected for the volume filtered and expressed as number of individuals per 1000 m^3. Spatial distribution of eggs and larvae was examined using contoured density plots generated with Surfer 6.0 for Windows 95. This contouring allowed interpolation, smoothed the variability inherent in ichthyoplankton sampling, and helped clarify spatial patterns.

Spatial analysis of the association between the egg and larval stages and the various environmental variables was explored using Pearson correlations with Bonferroni-adjusted p values. Seasonal trends were removed from all variables by calculating the residuals of a linear regression between each variable against day of year, and correlations were then based on residuals from this analysis. Six environmental variables were examined: temperature and salinity at 5 m, average temperature and salinity from 5 to 40 m, bottom depth, and chlorophyll a concentration.

Results

Coastal circulation and wind data

Of the 1000 drifters released, a total of 124 were recovered, and all were recovered northward of the islands (see Fig. 1). Drifters were recovered in the eastern channel (40% of total recoveries, all released from Perch Rock), the head of the bay (6.5% of total recoveries, all released from Bar Haven), and the western channel (53% of total recoveries,

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Fig. 2. (*a*) Along- and (*b*) across-bay currents from the eastern (solid line) and western (dashed line) sides of the bay. All data filtered with an eighth-order Butterworth lowpass filter and rotated to align with the bay.

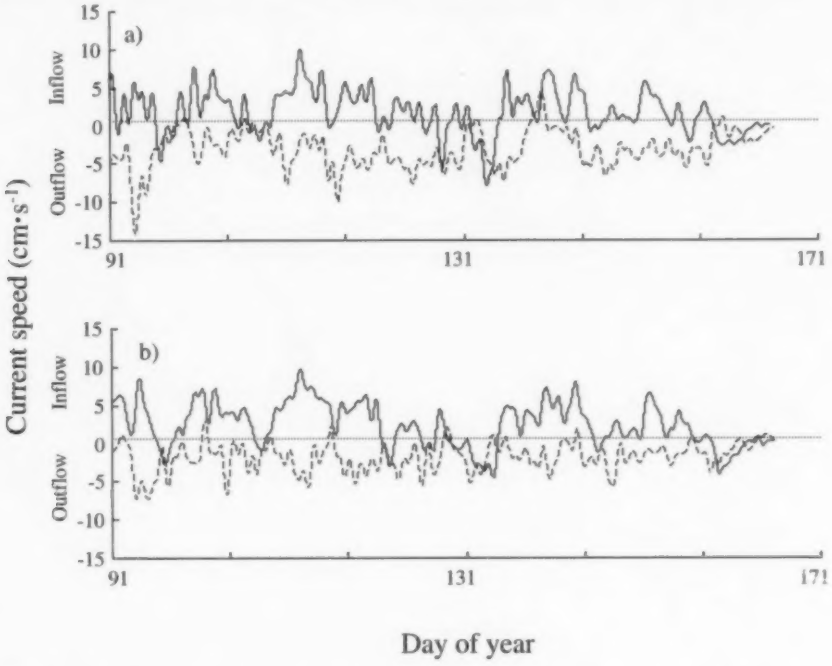
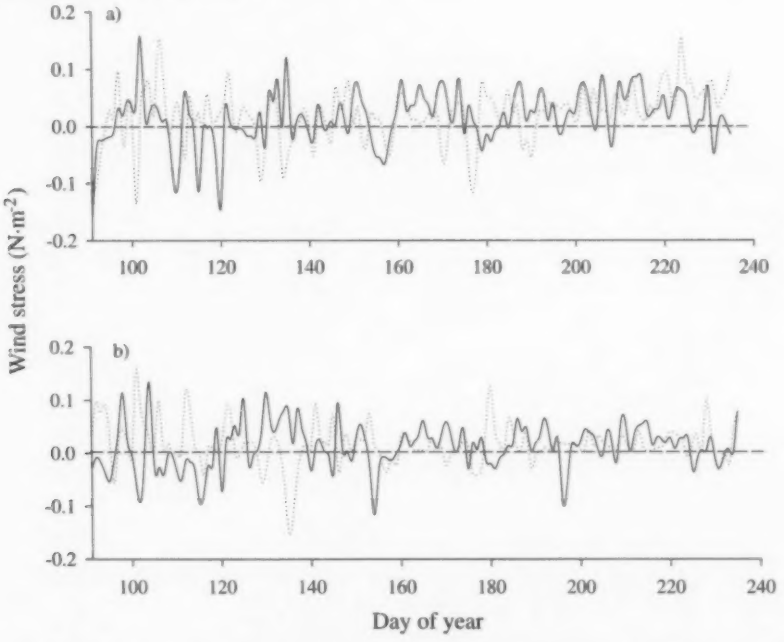


Fig. 3. (*a*) Along-and (*b*) across-bay wind stress from 1997 (solid line) and 1998 (dotted line). All data filtered with an eighth-order Butterworth lowpass filter and rotated to align with the bay.



5% released from Perch Rock and 95% released from Bar Haven).

Current measurements show that predominant currents on the eastern side were into the bay, whereas currents measured on the west were predominantly out of the bay (Fig. 2). The across-bay flow was also reversed between

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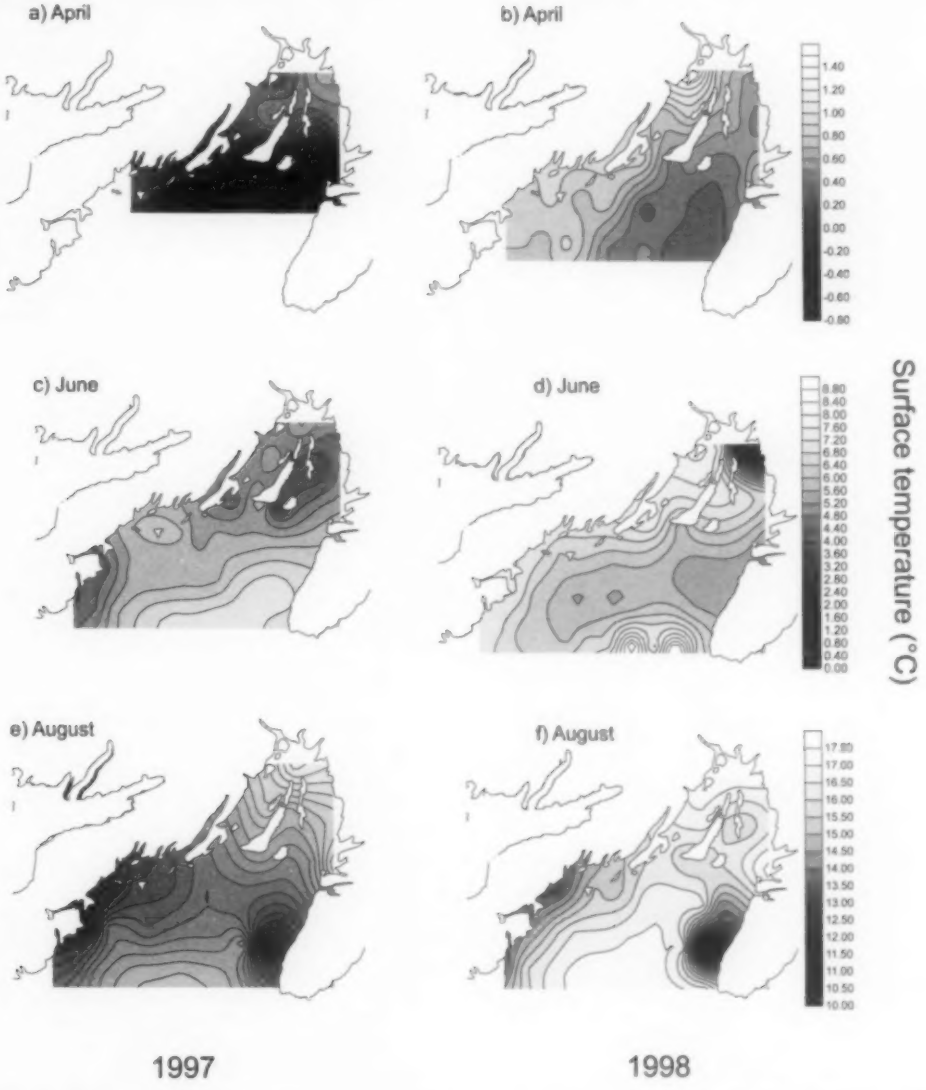
Fig. 4. Distribution of surface temperature (5 m) based on survey CTD profiles. Contours represent interpolations from the stations shown in Fig. 1.



sides of the bay, with flow predominately to the east on the eastern side and to the west on the western side. The average monthly along-bay and across-bay currents from the eastern side of the bay decreased gradually from April to June, whereas the currents from the western side did not show a similar change (Fig. 2). Cross-correlations of along-bay currents from the eastern and western sides of the bay gave significant correlations at both negative (positive correlation coefficients at lags of 13 and 14 days) and positive (negative correlation coefficients at lags of 6 and 7 days) lags.

Patterns in wind stress were similar for both years. The predominant winds were from the southwest and decreased in magnitude from spring through to early fall. The along-bay and across-bay components of the wind stress were also very similar between years, with the exception of a period of high offshore winds early in 1997 (Fig. 3). The means and standard deviations in the monthly along-bay and across-bay winds were consistent between years. Analysis of variance of the frequencies from Fourier transformations of the u and v components shows that although the 1997 and 1998 along-bay stresses were not significantly different ($F = 0.765$, $p = 0.993$, df = 3570), the across-bay stress frequencies did differ ($F = 8.581$, $p = 0.003$, df = 3466). Cross-correlations between the along-bay wind stress and along-bay currents were not significant for the western or eastern sides of the bay at positive lags from 0 to 18 days.

Environmental conditions

During April cruises, the bay was very homogenous, with <1°C increase from the northern to southern boundaries

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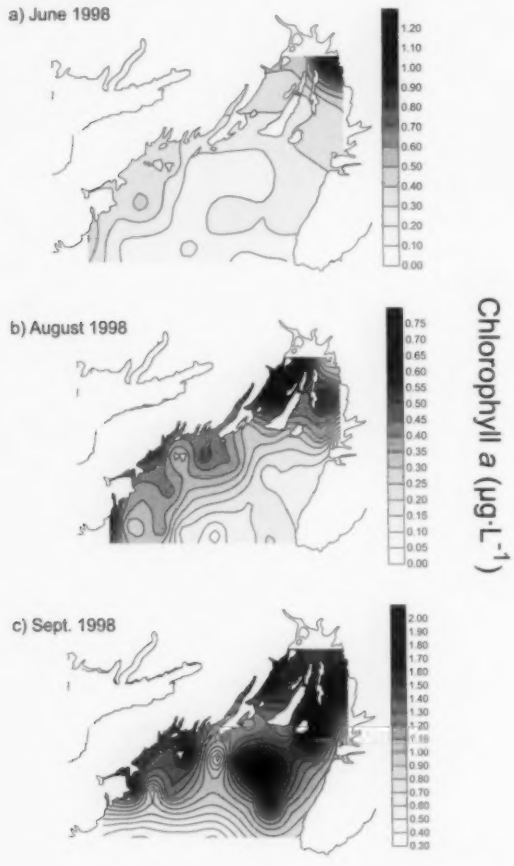
Fig. 5. Distribution of chlorophyll *a* (5 m) based on extractions from water samples taken during June, August, and September survey cruises. Contours represent interpolations from the stations shown in Fig. 1.



(Fig. 4). By June of 1997, there was evidence of localized upwelling as indicated by the presence of cold water associated with the islands. However, in 1998, this cold water was not observed, and the northeast part of the bay was characterized by cold water. Patterns in August of both years were very similar, with low temperatures on the southwestern side of the bay and near Perch Rock. There was also the suggestion of a decreasing gradient in surface and mixed layer temperatures from north to south. Between-year comparisons of surface and mixed layer temperature averages (upper 40 m) for the entire bay indicate a 1–2°C increase for corresponding time periods from 1997 to 1998.

The range in salinity (at 5 m) within a cruise was typically <1 psu and was largely invariant during the study periods (~31.5–31.9 psu) except for a Bar Haven station with 26.5 psu during April of 1998. Despite the modest range in salinity, a consistent gradient of increasing salinity was associated with the western side of the bay in both years, extending from the head of the bay to the southern boundary. This gradient was evident in June and August of 1997 and June of 1998 and is consistent with a freshwater inflow at the head of the bay and the entrainment of this water in the predominant southern flow along the western channel. Salinity measurements at the surface indicated no consistent between-year differences.

The vertical profiles of density show a slight stratification in June and a well-defined pycnocline by August of both years. By early fall (October 1997, September 1998) of both years, the pycnocline began to break down. Stability ($\Delta\sigma_t$ 5–40 m) values were higher in 1998, but an examination of the spatial pattern in stability during June of both years consistently showed an area of well-mixed water near the centre of the bay suggestive of increased vertical mixing. This well-mixed area near the centre was also observed in August 1997; however, the dominant feature at this time was a band of well-mixed water along the southwest coast of the bay.

The measurements of chlorophyll *a* concentrations taken using different instruments were addressed separately. The April 1997 survey that utilized a CTD-mounted fluorometer gave readings at 5 m ranging from 3.0 $\mu g \cdot L^{-1}$ in the head of the bay to 0.60 $\mu g \cdot L^{-1}$ near the mouth, with a general trend of decreasing algal biomass with increased distance out of the bay. Chlorophyll *a* measurements from the extractions indicate large variation from June to September (Fig. 5). In June and August 1998, relatively high concentrations were associated with the head of the bay (June and August) and the western side (August), with peak values ranging from 0.75 to 1.2 $\mu g \cdot L^{-1}$. The pattern evident in September 1998 was quite different from that of the spring and summer, with the peak in concentration of 2.8 $\mu g \cdot L^{-1}$ occurring in the centre of the bay. These spatial and temporal patterns in chlorophyll *a* were consistent with the distribution of chlorophyll *a* observed in SeaWiFS (Sea-viewing Wide Field-of-view Sensor) satellite images for the corresponding time period (images supplied by L. Pazzant, Bedford Institute of Oceanography, Dartmouth, N.S.).

Ichthyoplankton

The difficulty in separating early-stage Atlantic cod eggs from those of witch flounder, *Glyptocephalus cynoglossus*, and haddock, *Melanogrammus aeglefinus*, necessitated the Atlantic cod – haddock – witch flounder grouping (e.g., Brander and Hurley 1992). Nonetheless, careful identification of many late-stage eggs and larvae indicated only incidental numbers of these other species, and it is therefore reasonable to assume that the majority of sampled Atlantic cod – haddock – witch flounder eggs were Atlantic cod (>99%).

Temporally, densities of stage I eggs were highest in April of both years (particularly April 1997), suggesting associated spawning peaks. Spatially, the highest densities of stage I eggs were observed at stations near Bar Haven, Perch Rock, and Oderin Bank (Fig. 6), coincident with adult Atlantic cod spawning aggregations (Lawson and Rose 2000). Late-stage eggs usually occurred on the western side of the outer bay where eggs spawned within the bay would have had the longest development times, given the spawning locations and predominant currents in the bay. The temporal peak in late-stage eggs was observed in May of 1997 (stages III and IV) but occurred in June (stage III) and August (stage IV) of 1998. The average densities of late-stage eggs

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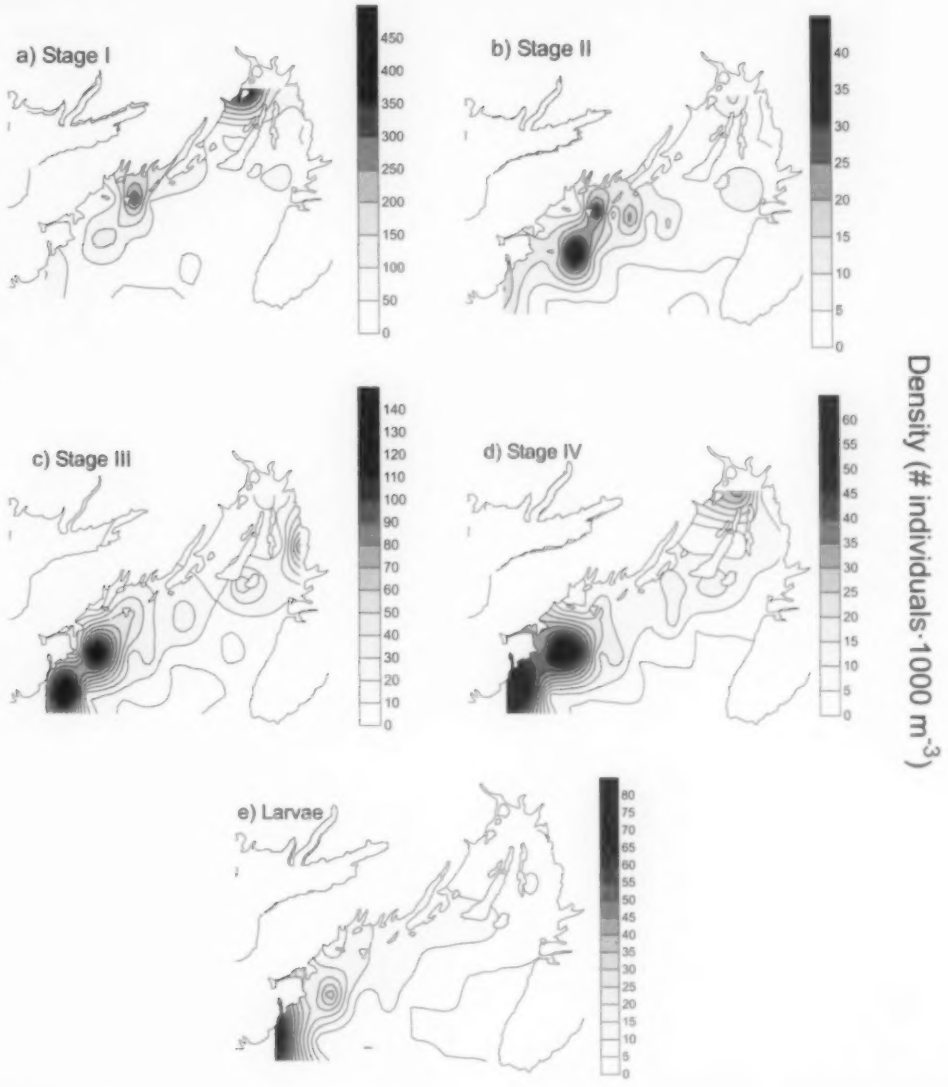
Fig. 6. Distribution of Atlantic cod eggs and larvae sampled during August 1998. Contours represent interpolations from the stations shown in Fig. 1.



(stages III and IV) and larvae were higher in 1998 than in 1997 (Fig. 6).

Larval densities were highest on the southwestern side of the outer bay (Fig. 6), corresponding to the pattern observed in late-stage eggs. Although egg densities in April 1997 were higher than at any other time in the two years of the study, larval densities were very low in all of 1997 and highest in August of 1998 (Fig. 7). Back-calculations of the spawning period expected to result in these peaks in larval density (represented as hatched bars in Fig. 7) were based on mixed layer water temperatures and temperature-dependent development relationships (see Pepin et al. 1997). The range of larval ages was based on the 95% confidence intervals for the larval lengths of individuals collected during each cruise and a 0.33 mm·day^{-1} growth rate from Pepin et al. (1995). This calculation predicts that the spawning activity that produced the observed hatching likely occurred in mid-March and early July for 1997 and in mid-July for 1998.

Examination of the density of stage I eggs at each of the identified spawning grounds (Fig. 8) shows similar between-year patterns at Bar Haven, peaking in April and decreasing in subsequent months. In contrast, Oderin Bank eggs peaked in June of 1997 and in August of 1998.

In most of the samples taken, the numbers of Atlantic cod larvae sampled were too low to make any inferences about size structure. However, in August of 1998, a total of over 900 Atlantic cod larvae were sampled, in contrast with a total of 41 larvae in all of 1997. Figure 9 shows mean (±SD) larval length measured for each transect. The pattern shows increases in larval size from the inner to the outer bay, a pat-

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Fig. 7. (*a*) Seasonal changes in mean Atlantic cod stage I egg density for 1997 (open circles) and 1998 (solid circles). The dashed line shows the predicted date when eggs spawned in the April peak would be expected to hatch. (*b*) Seasonal changes in mean larval density for 1997 (open circles) and 1998 (solid circles). The dashed line is as defined in Fig. 7*a*. Arrows link peaks in larval density to the predicted period in which spawning would have to occur to produce the larvae sampled at each peak larval density (hatched bars). Back-calculation of the spawning period was based on relationships in Pepin et al. (1997) and a maximum larval life based on 95% confidence intervals of larval lengths and a 0.33 mm·day^{-1} growth rate (Pepin et al. 1995). The number of larvae was 30 in August 1997 and 821 in 1998.

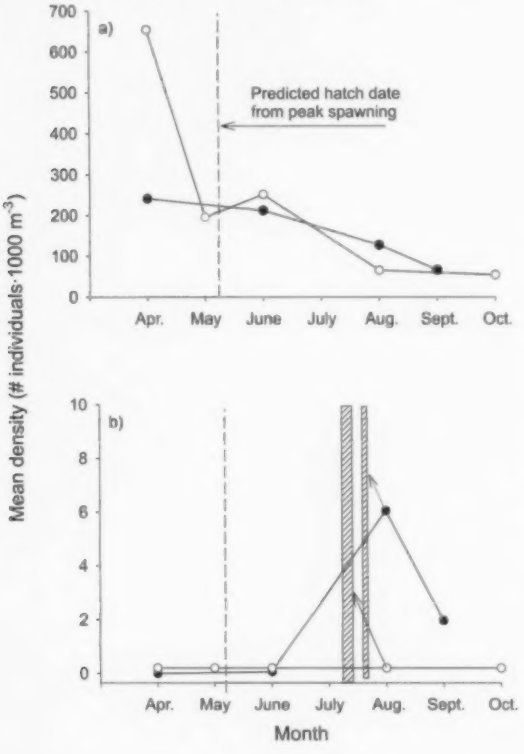


Fig. 8. Density of stage I Atlantic cod eggs at each of the spawning sites for (*a*) 1997 and (*b*) 1998. Open bars, Bar Haven; hatched bars, Oderin Bank; solid bars, Perch Rock. See Fig. 1 for location of sites. No data from Perck Rock for April in either year.

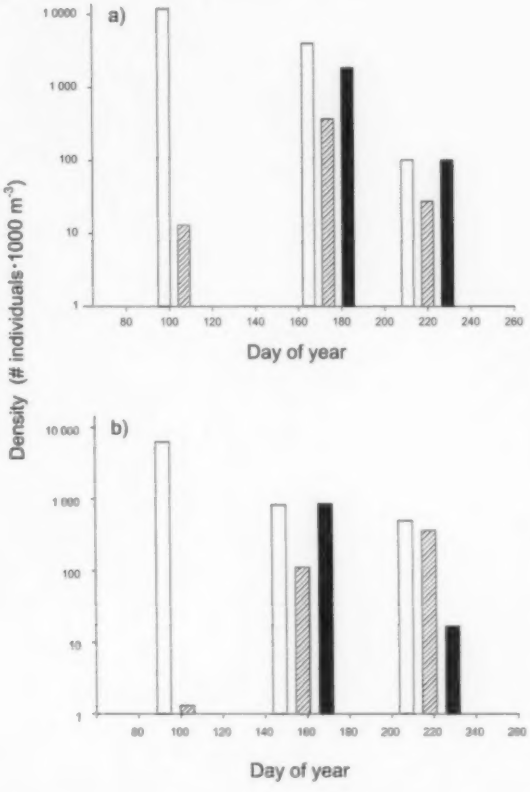
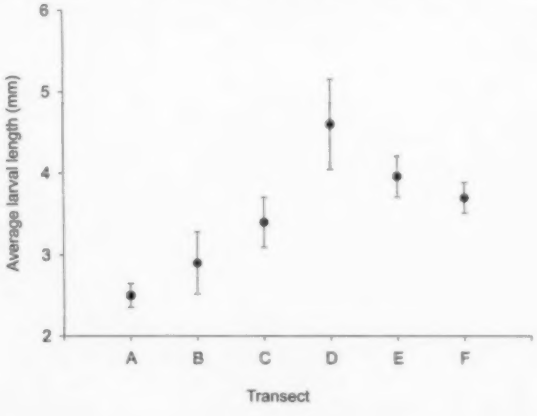


Fig. 9. Changes in Atlantic cod larval size with transect based on data collected during Tucker trawl surveys. Lengths are averaged across each transect, and error bars denote ±1 SD.



tern consistent with predicted coastal flow patterns as larvae develop and are transported south along the western side of the bay. There was also a significant decrease in larval length between August and September 1998 (GLM, F = 28.077, $p < 0.001$, df = 1070)

Correlations between environmental variables and ichthyoplankton are given in Table 1. Although there is a lack of a consistent pattern, surface salinity more than any other variable was significantly correlated with egg and ichthyoplankton densities.

Discussion

One of the challenges for marine fish is to place reproductive propagules into an environment where they are likely to hatch and settle (Harden Jones 1969). There is increasing evidence that Atlantic cod spawning occurs in multiple coastal areas of Newfoundland (Smedbol et al. 1998; Lawson and Rose 2000) and that these areas may be characterized by higher survival and growth rates (Pepin et al.

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Table 1. Atlantic cod egg density (separated by stage) and environmental variable correlations.

	Cruise date							
Stage	Apr. 1997	May 1997	June 1997	Aug. 1997	Apr. 1998	June 1998	Aug. 1998	Sept. 1998
I				–d, –ss, –ms	+st, –ss, –ms	+ch, +st, +mt, –ss, –ms	–d, +ch	–d
II		–d				+ch, +mt		
III		–d, +st, +mt		–ss				
IV	+mt			–ss, –ms	+st, +mt		+ch	–ss
Larvae				–st		+ch, –ss, –ms		

Note: d, bottom depth; ch, chlorophyll *a*; st, surface temperature; mt, mixed layer temperature; ss, surface salinity; ms, mixed layer salinity. Only significant correlations (adjusted *p* values) are reported. A negative sign indicates a negative correlation and a plus sign indicates a positive correlation. Sign tests identified depth with stages I and III as well as mixed layer salinity with stage III as significantly ($\alpha = 0.05$) negative.

1995) than offshore habitats. But whether coastal spawning results in a habitat advantage for juveniles depends on successful hatching near the coastal habitat, which in turn depends on timing and location of spawning in relation to variables such as water temperature and surface currents.

Spawning location

During the two years of our study, stage I eggs were concentrated in three areas: Perch Rock, Bar Haven, and Oderin Bank. The pattern in these early-stage eggs suggests that most of the spawning activity in Placentia Bay was confined to these sites and the majority of Atlantic cod eggs and larvae that we collected in the bay were produced by these spawning aggregations. Although the duration of stage I eggs is on the order of 1–2 weeks in water temperatures typical of April (see Pepin et al. 1997), by June and August the stage I duration would be on the order of days, thus suggesting limited drift and a greater likelihood that spawning occurred near where eggs were collected in highest abundance. These same inshore locations in Placentia Bay have been identified by Lawson and Rose (2000) as major spawning sites based on acoustic biomass surveys and samples collected to evaluate gonadal condition.

Although spawning may occur broadly on offshore shelf regions south of our study area (Myers et al. 1993), it is unlikely that offshore spawning contributes to egg and larval pattern within Placentia Bay. Late-stage eggs and larvae are largely absent from the eastern side of the bay and are consistently associated with the head or western side of Placentia Bay where flow is predominately out of the bay. Moreover, Atlantic cod eggs and larvae were effectively absent in a series of samples collected from the shelf south of Placentia Bay in June of 1998 (I.R. Bradbury, P.V.R. Snelgrove, and S. Fraser, unpublished data). From our data, it is impossible to predict how important inshore spawning is for south coast Newfoundland Atlantic cod recruitment, but it is clear that substantial inshore spawning occurs at spatially consistent sites in Placentia Bay and that these spawning events are responsible for most of the Atlantic cod reproductive propagules that are observed within the bay. The reasons for the repeated use of these discrete spawning areas by Atlantic cod are unclear, but chlorophyll data and SeaWiFs imagery for spring and summer indicate that algal biomass is usually highest at the head and western side of the bay. This increased algal biomass, although admittedly a very crude indication of available carbon, may translate into a more favourable feeding habitat for adults, and in addition, it may provide greater food resources for hatching larvae (Cushing 1990). This hypothesis is, however, speculative without additional data linking algal production to zooplankton production and subsequently to larval feeding conditions.

Timing of spawning

Perch Rock and Bar Haven egg numbers were highest early in the spring sampling period and declined through the summer. In 1998, Bar Haven egg numbers dropped off less precipitously than in 1997, so that a greater proportion of eggs were spawned in August of 1998 in comparison with 1997. Additional "late" egg production was evident at Oderin Bank in 1998, where peak egg numbers were observed in August. The delay in egg production at Oderin Bank is consistent with direct observations of adult spawning in the area (Lawson and Rose 2000). Reasons for the delay in spawning in 1998 remain unclear, particularly given that water temperatures were warmer than in 1997. Regardless of the reason, the increase in egg production later in the spawning season in 1998 resulted in higher numbers of eggs spawned in warmer water (a difference of 8.65°C compared with the April 1997 spawning peak). Thus, the delay in spawning likely led to faster development times (from 18.9 days to <7 days, relationships from Pepin et al. 1997) and much shorter dispersal distances prior to hatching.

Egg and larval transport

The circulation data suggest an embayment with mean cyclonic currents moving northeast along the eastern side and to the southwest along the western side, exiting the bay near the southwest boundary. There is some suggestion (Lawrence et al. 1973) that there may be less directionality to the flow patterns in the head of the bay and near the centre of the outer bay, but our results suggest that flow at the periphery is generally strong and consistent in direction.

The lack of a strong interaction between wind stress and current patterns in Placentia Bay is unexpected. Similar studies of coastal circulation have been conducted in other Newfoundland bays and provide a useful comparison (e.g., Trinity Bay (Yao 1986), Conception Bay (deYoung et al. 1993)). In Trinity Bay, a major part of the near-surface current variance could be accounted for by along-bay wind stress. However, in early summer when cyclonic flow patterns were observed, the currents were not coherent with the wind, and nonlocal forcing was suggested as a possible explanation (Yao 1986). The importance of nonlocal forcing has also been demonstrated for Conception Bay (deYoung et al. 1993)). The observation that the current variance could not be explained using along-bay wind stress when predomi-

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nant flow patterns in Placentia Bay and Trinity Bay were similar suggests the influence of nonlocal forcing in Placentia Bay. Moreover, the strong correlation between the along-bay currents on the eastern and western side of Placentia Bay suggests continuous flow moving around the bay and is inconsistent with the patterns expected due to wind forcing.

The location of the major freshwater source in Placentia Bay is just north of Bar Haven and allows utilization of salinity as a passive tracer of predominant drift patterns. The consistent significant correlations between surface salinity and egg and larval densities suggest similarities in pattern formation. These correlations, as well as the salinity gradient along the western coast, provide further support for the predominant drift out of the bay on the western side.

Although early-stage eggs were concentrated near the spawning grounds, peaks in late-stage eggs (III and IV) were observed on the western side where predominant flow exits the bay. The highest larval densities were also sampled in this area near the mouth of the bay on the western side, generally to the south of the late-stage eggs. The location of the highest larval densities coincided with the areas of highest algal abundance and may have significant consequences for larval survival (Cushing 1990). Consistent with this hypothesis is the fact that the largest mean larval lengths in August 1998 were observed in areas of elevated algal biomass. However, because patterns in larval length do not closely reflect patterns of algal biomass when the entire bay is considered, it is more likely that larger size reflects older individuals and longer transport times rather than a linkage with higher food availability.

Seasonal and year-to-year trends

The seasonal variation observed in the environment, coupled with the extremely protracted spawning period, suggests the potential for large, environmentally driven seasonal variation in egg and larval spatial and temporal distributions, development, and retention. Although peaks in mean egg density were observed in April of both years, relatively high larval densities were only observed in late summer (August 1997 and 1998, September 1998). Based on temperature-dependent development rates (Pepin et al. 1997), eggs spawned during April should have hatched around mid-May and been observed in May (1997) and June (1997 and 1998). The absence of larvae at these times suggests high loss rates either through flushing from the bay or through reduced survival. The lack of larvae from the peak in egg abundance is in contrast with the presence of large numbers of larvae in August samples when egg production was low.

There are several possible reasons for the seasonal variation in hatch rates that generally assume either differences in resultant transport or differential survival. Seasonal differences in transport rates could account for differences in hatch rates; however, there is no evidence from either the wind stress or the current data to suggest that this was the cause. Seasonal variation in location of spawning relative to reduced flow speeds could result in variation in hatch rates within the bay. Spawning locations, however, as indicated from the stage I egg distribution, were consistent within years, suggesting that spatial variation in spawning could not explain differences in hatch rates.

Differential survival can be caused by increases in either starvation or predation. The most common phenomenon related to larval starvation and foraging success is a match or mismatch to the annual spring algal bloom (Cushing 1990). In 1998, SeaWiFs data allowed us to examine the timing and duration of the spring algal bloom, which occurred in early April and lasted only about 2–3 weeks. The highest larval densities were observed in August and were clearly decoupled from the spring bloom, suggesting minimal potential for influence. Similar observations made by Gagné and O'Boyle (1984) for the Laurentian Channel area suggest extensive Atlantic cod spawning in summer despite spring and fall algal blooms.

The most plausible explanation for the seasonal differences in hatch rates is that environmental effects on the egg vital rates resulted in faster development (less than the residency time) during the summer months and resulted in increased hatching within the bay. Therefore, flushing of Placentia Bay by the relatively strong cyclonic flow could limit the occurrence of larvae to warm months when time to hatch does not exceed residency time. This theory is consistent with the spatial pattern observed in both the eggs and larvae. The dramatic increase in larval abundance in 1998 relative to 1997 could then be attributed to increased egg production late in the spawning season.

The idea that temporal variation in recruitment in species with planktonic eggs and larvae is dependent on advective loss of individuals from the area has been termed the "member-vagrant" hypothesis (Sinclair 1988). Advection out of a population's range or away from nursery grounds may not necessarily result in mortality; nonetheless, these propagules may be effectively lost to the population due to their spatial separation. Our results suggest an extension of both the member-vagrant (Sinclair 1988) and the right site hypotheses (deYoung and Rose 1993). We suggest that in coastal areas where spawning appears to recur, temporal variation in spawning, which influences development times and retention, may produce similar consequences. We hypothesize that slow development rates as a consequence of spawning at cold water temperatures result in increased vagrancy from the local population.

Although this study is restricted to the egg and larval stages, a corresponding study of juvenile Atlantic cod spatial and temporal pattern in Placentia Bay has been ongoing. Robichaud and Rose (1999) observed a fivefold increase in juvenile Atlantic cod catch rates from 1997 to 1998, suggesting a strong agreement with the larval densities. The link between recruitment and larval densities has been made by Bjorke and Sundby (1986), who observed a significant correlation between Atlantic cod larval numbers and year-class strength. If larval abundances can be interpreted as indicators of future recruitment (as similarities between the patterns in larval fish observed here and juvenile patterns suggest), then late spawning may be critical to successful recruitment within Placentia Bay.

The eventual fate of eggs and larvae advected from the bay remains unresolved. Offshore data from June of 1998, when late-stage eggs and larvae would have been present if offshore survivorship was high, indicated very low densities of early stages and an absence of late-stage eggs and larvae (I.R. Bradbury, P.V.R. Snelgrove, and S. Fraser, unpublished

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data). Moreover, recent studies of the genetic (Ruzzante et al. 1996) and behavioural (Wroblewski et al. 1996) aspects of coastal Atlantic cod populations suggest the occurrence of distinct subpopulations. If these subpopulations do indeed exist, then the potential for gene flow between the populations of inshore and offshore Atlantic cod may be relatively small and survivorship of eggs advected offshore may be poor. A comprehensive survey examining both the inshore and offshore areas throughout the spawning season is needed to address this question. Our data suggest that densities of early-stage eggs may be a poor indicator of subsequent larval abundance in coastal Newfoundland. Specifically, because timing is more important than number of eggs spawned, peaks in inshore spawning may be less important to larval production when they occur in early spring. It may be the late spawners that contribute most to successful hatching of larvae and to subsequent inshore settlement and recruitment.

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Modeling the potential effects of climate change on leaf pack processing in central Appalachian streams

Karen M. Buzby and Sue A. Perry

Abstract: A computer simulation model was constructed to evaluate some of the projected impacts of climate change, including elevated temperatures and increased frequency and magnitude of floods and droughts, on leaf pack processing in central Appalachian streams. The model simulated microbial processing, invertebrate consumption, and transport along a 1-km second-order stream. We examined the effects of wet and dry years with and without a 2°C temperature increase. Both invertebrates and microbes processed more leaf material under the elevated temperature scenarios; however, the invertebrate response was greater than the microbial response. In the model, microbial processing is represented as a linear function of temperature; a nonlinear response might produce different results. Invertebrates processed a greater percentage of the inputs in wet than in dry years, while microbial processing rates were unaffected. A 20-year flood event occurring in November, January, or March caused more than 50% of the leaf inputs to be exported, leaving little detrital material available for invertebrate consumption. The timing of the flood event made little difference to the simulation results. All climate change scenarios resulted in decreases, sometimes substantial, in coarse particulate organic matter availability to shredders during the summer months.

Résumé : Nous avons construit un modèle de simulation informatique pour évaluer certains des effets prévus du changement climatique, notamment l'élévation de la température et l'augmentation de la fréquence et de l'ampleur des crues et des sécheresses, sur la transformation de la couche de feuilles dans des cours d'eau du centre des Appalaches. Le modèle simulait la dégradation par les bactéries, la consommation par les invertébrés et le transport sur un tronçon de 1 km d'un cours d'eau de 2e ordre. Nous avons examiné les effets des années sèches et des années humides avec et sans une hausse de la température de 2°C. Les invertébrés comme les microbes transformaient davantage de matière foliaire dans le scénario de température plus élevée, mais l'intervention des invertébrés était toujours plus importante que celle des microbes. Dans le modèle, la transformation microbienne est représentée par une fonction linéaire de la température; une réponse non linéaire pourrait produire des résultats différents. Les invertébrés transformaient un plus gros pourcentage des apports les années humides que les années sèches, tandis que le taux de transformation par les microbes était inchangé. Un événement de crue à récurrence de 20 ans, survenu en novembre, janvier ou mars, causait l'exportation de plus de 50% des apports de feuilles et laissait peu de matière détritique à consommer pour les invertébrés. Le moment de survenue de la crue avait peu d'effet sur les résultats de la simulation. Tous les scénarios de changement climatique se traduisaient par des baisses, parfois substantielles, de la disponibilité de la matière particulaire grossière pour les déchiqueteurs pendant les mois d'été.

[Traduit par la Rédaction]

Introduction

Streams draining forested watersheds derive most of their energy from detrital sources, either directly through leaves, sticks, and flowers falling into the stream or indirectly as dissolved organic carbon entering in the groundwater (Fisher and Likens 1973). Changes in environmental conditions that affect detrital processing rates have the potential to change the productivity of headwater streams, where most of the leaf litter is initially processed, and of larger streams, which rely on a steady supply of processed fine particulate organic matter (FPOM) from upstream sources.

Climate change due to anthropogenic release of greenhouse gases, with its predicted 1.5–4.5°C rise in air tempera-

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ture (Schneider 1993; Moore et al. 1997) and concurrent increases in water temperature, is likely to impact detrital processing rates. It is generally believed that the increased temperatures expected with climate change will increase processing rates; however, the extent of the increase and the ramifications of increased processing rates are unknown (Allan 1995). Temperature increases are expected to be greater in winter than in summer (Moore et al. 1997) and as such might be expected to have greater impacts on detrital processing than on other ecosystem functions. In addition to predictions regarding changes in air and water temperature, changes in precipitation patterns and forms of precipitation due to climate change have been hypothesized. It is generally believed that there will be an increase in the variability of stream flow and an increase in the frequency of both floods and droughts (Arnell et al. 1996).

There are also potential indirect effects of climate change on leaf processing in streams. Nutrient concentrations in stream water, which may be altered due to the effects of climate change on the terrestrial environment (Meyer and Pulliam 1991), have been shown to affect rates of leaf processing (Meyer 1980). Changes in riparian vegetation may alter the magnitude of leaf inputs as well as their quality (Meyer and Pulliam 1991; Sweeney et al. 1991). Sweeney et al. (1991) voiced concern that the rapidity and magnitude of the predicted temperature increases may cause extirpation or extinction of some aquatic insect species, some of which are important in leaf processing (Anderson and Sedell 1979).

Predicting the changes in decomposition dynamics due to climate change is difficult due to the interactions between microbes and invertebrates, the uncertainty regarding the response of invertebrate communities to increased temperatures, as well as the unknown implications of altered discharge regimes (Allan 1995). Simulation modeling is a tool that can be used to identify the more likely ecosystem responses as well as suggesting where additional information is required to more accurately predict system response.

We constructed a simulation model to evaluate some of the potential impacts of climate change on leaf litter processing in small streams of the central Appalachians. We used the model to examine the influence of wet and dry years with and without elevated temperatures as well as the impact of large storm events on leaf pack processing. The model simulates microbial processing as functions of initial substrate quality and stream temperature and invertebrate processing as functions of initial substrate quality, length of time that the detrital material was present in the stream, and stream temperature. Export is simulated as a function of peak daily discharge. Literature values were used for microbial processing rates, but invertebrate feeding rates and exports were determined as part of model development. The model predicts the amount of leaf inputs processed by microbes and invertebrates and losses due to exports during storm events. We did not address how changes in substrate quality and quantity might affect invertebrate growth or survival. No attempt was made to model FPOM or dissolved organic carbon dynamics.

Our model is similar to one developed by Webster (1983) but does have some major differences, primarily in the way in which processing of leaves in different quality categories was handled. We retained the leaf processing categories rather than aggregating all inputs into one state variable. The other major difference between the models concerns the way in which invertebrate feeding rates were treated. We used taxon-specific feeding rates modified by temperature, initial leaf quality, and degree of conditioning rather than using the same feeding rate modified by stream temperature for all invertebrates. Maintaining processing categories as well as more specific feeding relationships enabled us to make predictions regarding changes in species composition of the canopy or the invertebrates.

Methods

Study site

Measurements at Wilson Hollow, a second-order stream in the Fernow Experimental Forest (FEF) (U.S. Forest Service), were used to generate many of the parameters used in the simulation model. The FEF is located in the central Appalachian Mountains of north-central West Virginia. It is characterized by steep forested slopes and narrow valleys. Vegetation is dominated by red oak (*Quercus rubra*), tulip poplar (*Liriodendron tulipifera*), and sugar maple (*Acer saccharum*). A more complete description of the FEF may be found in Adams et al. (1994).

Wilson Hollow, draining 131 ha, is formed by the confluence of two first-order streams draining watersheds 7 (WS7) and 13 (WS13). Approximately 100 m downstream, it is joined by a third first-order stream draining watershed 6 (WS6). Total stream length is 1012 m. Discharge records were available for WS6 and WS7 since 1956, for WS13 since 1988, and for Wilson Hollow since 1992. Stream flow is highest during the winter and early spring. First-order streams are often dry in late summer and early autumn, and occasionally, Wilson Hollow is dry during August and September. Air and stream temperature records for varying lengths of time were also available.

Model description

We constructed a simulation model to examine the consequences of climate change on leaf processing in small streams of the central Appalachians. A flowchart outlining the basic processes in the model is shown in Fig. 1. The model is written in Fortran and simulates processes on a daily basis (the computer code is available from K.M.B. upon request). The simulation runs from August 15 through June 29. We did not simulate an entire year because these streams often go dry during the late summer and early fall, which changes the decomposition dynamics. A description of the equations and parameters that we used follows.

Physical parameters

The model required daily inputs of stream temperature. Two years of daily stream temperature data were available; however, they did not encompass the variability in conditions that we wanted to explore. There were, however, more than 40 years of air temperature data available. To extend the stream temperature record, we used the following regression:

$$\text{Stream temperature} = 0.50 \times \text{mean}_{\text{7-day minimum}} + 7.21$$

where $\text{mean}_{\text{7-day minimum}}$ is the mean of the previous 7 days' minimum air temperature ($r^2 = 0.89$ between calculated values and data). Estimated stream temperatures during the simulation period ranged between 0.4 and 16.2°C.

The model also required daily inputs of peak discharge from WS7 and WS13, the two streams that join to form Wilson Hollow. Although an extensive data set exists for much of the FEF, dis-

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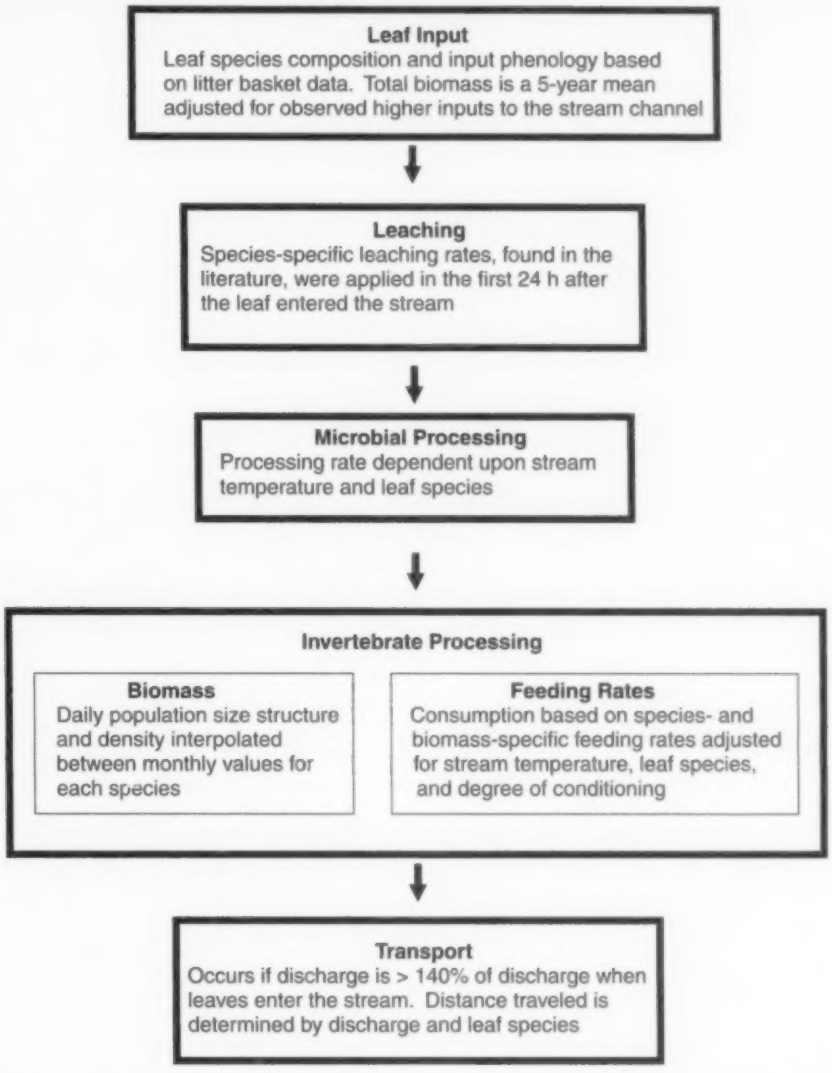
Fig. 1. Model operations that occur in each 1-m section of stream channel.


Leaf Input
Leaf species composition and input phenology based on litter basket data. Total biomass is a 5-year mean adjusted for observed higher inputs to the stream channel
Leaching
Species-specific leaching rates, found in the literature, were applied in the first 24 h after the leaf entered the stream
Microbial Processing
Processing rate dependent upon stream temperature and leaf species
Invertebrate Processing
Biomass
Daily population size structure and density interpolated between monthly values for each species
Feeding Rates
Consumption based on species- and biomass-specific feeding rates adjusted for stream temperature, leaf species, and degree of conditioning
Transport
Occurs if discharge is > 140% of discharge when leaves enter the stream. Distance traveled is determined by discharge and leaf species

charge records for WS13 were only available since 1988. We used the following regression equation to relate the two watersheds ($r^2 = 0.96$):

$$\text{Peak discharge}_{WS13} = 0.6774 \times \text{peak discharge}_{WS7} - 0.03662.$$

Discharge was calculated at 1-m intervals along Wilson Hollow from below the weir pond on WS7 (180 m above the confluence of WS7 and WS13) to above the weir at the base of Wilson Hollow. Except for the inputs at the confluence of WS6 and Wilson Hollow, increases in discharge were attributed to groundwater inputs. The following equations were used to calculate discharge at each 1-m interval:

$$\text{Discharge in the first 180 m} = \text{Peak discharge}_{WS7}(0.0021 \times \text{distance downstream})$$

$$\text{Discharge from 181 to 290 m downstream} = (\text{Peak discharge}_{WS7} + \text{peak discharge}_{WS13})(0.0021 \times \text{distance downstream})$$

$$\text{Discharge below 290 m} = ((2 \times \text{Peak discharge}_{WS7}) + \text{peak discharge}_{WS13})(0.0021 \times \text{distance downstream}).$$

Channel width remains constant except where other streams join Wilson Hollow. Initial channel width is 2.0 m, which increased to 2.7 m at the confluence of WS7 and WS13 and increased further to 4.7 m where WS6 joins Wilson Hollow.

Leaf inputs

Species composition, phenology, and magnitude of overhead

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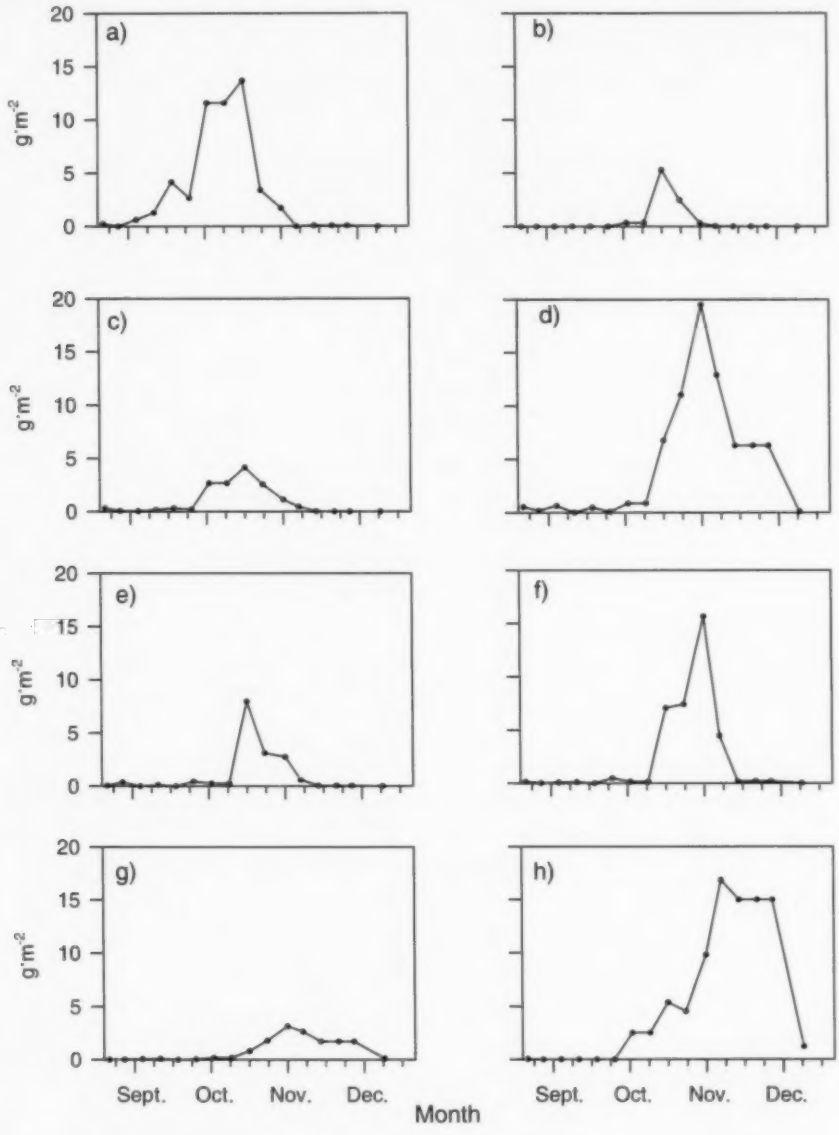
Fig. 2. Overhead litter inputs to Wilson Hollow in 1995. The eight species (*a*) basswood, (*b*) white ash, (*c*) American hornbeam, (*d*) tulip poplar, (*e*) black birch, (*f*) sugar maple, (*g*) American beech, and (*h*) red oak used in the simulation contribute more than 85% of the litter inputs.



leaf litter inputs were determined empirically. Overhead leaf inputs to Wilson Hollow were collected at mostly weekly intervals during fall 1995 from four 0.37-m^2 box collectors. The magnitude and timing of litter fall from the predominant species are shown in Fig. 2. Eight tree species, tulip poplar, red oak, black birch (*Betula lenta*), basswood (*Tilia americana*), white ash (*Fraxinus americana*), sugar maple, American beech (*Fagus grandifolia*), and American hornbeam (*Carpinus caroliniana*), were used in the simulation. The processing categories and relative biomass contribution of each species are given in Table 1. Values for the magnitude of blown-in material in proportion to overhead inputs and the timing of blown-in inputs were taken from Webster et al. (1990). Leaf inputs were assumed to cover the stream channel evenly.

Leaf transport

Leaf transport was simulated using species-specific regressions between discharge and distance traveled derived from data collected within the study reach (K.M. Buzby and S.A. Perry, unpublished data). In the model, transport occurs when discharge exceeds 140% of the discharge when the leaf material falls into the stream channel. We reasoned that the discharge when the leaf falls into the stream determines where within the water column the leaf is retained. It is unlikely to be transported downstream until discharge increases sufficiently to dislodge it. Although arbitrary, this assumption predicted actual leaf transport to within 0.8 m after 5 days and one storm event and to within 3 m after 5 days and three storm events in two separate transport experiments.

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Table 1. Species of leaves used in the simulation, percentage of total inputs, and processing category.

Species	% of total inputs	Processing category
Red oak	25.14	Slow
Tulip poplar	20.22	Medium
Basswood	12.62	Fast
Sugar maple	10.44	Medium
Black birch	4.40	Medium
American beech	4.33	Slow
American hornbeam	4.19	Medium
White ash	2.43	Fast

Under normal flow conditions, this relationship works well; however, it does not accurately portray leaf transport under conditions of high discharge (Snaddon et al. 1992). At some point, the water level is high enough that most of the water column is not in contact with potential retention surfaces such as sticks and rock faces. At this point, retention efficiency of the stream drops drastically. We had no data as to what discharge this would occur at and arbitrarily decided that if discharge exceeded a 50-year storm event (Helvey and Kochenderfer 1988), all leaf material would be exported.

Leaching

All soluble components were assumed to have been leached within the first 24 h. Leaching coefficients for red oak, sugar maple, and American beech were taken from Kaushik and Hynes (1971). We used the leaching coefficients for European white birch (*Betula verrucosa*) of Nykvist (1961) for both black birch and American hornbeam, the leaching coefficients of Griffith et al. (1995) for tulip poplar, and coefficients from Petersen and Cummins (1974) for white ash.

Microbial processing

Information regarding microbial processing, distinct from invertebrate processing, is scant. We used the category-specific microbial processing rates reported in Webster (1983) from a stream at Coweeta, North Carolina, treated by methoxychlor to eliminate invertebrates. Stream temperatures are slightly higher in Coweeta than in the FEF (annual means of 12.9 and 9.2°C, respectively); however, because the rates are calculated on a degree-day basis rather than on a daily basis, they should not overestimate microbial processing.

Invertebrate processing

In the model, we simulated the processing activities of five invertebrate taxa: the crayfish *Cambarus bartoni*, the stoneflies *Leuctra* spp. and *Peltoperla* sp., the caddisfly *Pycnopsyche* sp., and the cranefly *Tipula abdominalis*. These five taxa contribute more than 85% of the shredder production in WS4, a nearby undisturbed watershed in the FEF (Griffith et al. 1994). Daily invertebrate density and mean individual biomass were interpolated from monthly data collected to determine production in WS4 (Griffith et al. 1994). These values may represent an overestimate of shredder biomass because they are from a first-order stream rather than a second-order stream.

Invertebrate processing rates are determined by the interaction of four factors: invertebrate species, initial substrate quality, degree of conditioning of the leaf material, and stream temperature. The quantity of feeding rate data available for each of the invertebrate taxa used in the simulation was different; therefore, feeding rates were simulated to varying levels of precision. *Pycnopsyche*, *Peltoperla*, and *Leuctra* processing rates were modified by initial substrate quality, degree of conditioning, and temperature. *Tipula* rates were modified by temperature, and the processing rate used for *Cambarus* was constant.

Consumption rates for *Pycnopsyche* and *Peltoperla* were determined using an environmental chamber, which maintained temperature at either 5 or 10°C (±0.2°C) with a 12 h day : 12 h night cycle. Feeding substrates were air-dried leaf material that had been conditioned for 2–13 weeks in the weir pond at the base of Wilson Hollow. After conditioning, the leaf material was gently washed, placed in a zip-lock plastic bag, and frozen until needed. A similar procedure was used by Perry et al. (1987).

Equations relating biomass and consumption rate of *Pycnopsyche* are shown in Table 2. Consumption rates of dogwood were used for "fast"-processed leaves (sensu Petersen and Cummins 1974), consumption of sugar maple for "medium"-processed leaves, and consumption of red oak for "slow"-processed leaves. Consumption rates increase with increasing temperature. We approximated this increase by determining the difference in *Pycnopsyche* consumption rates of conditioned sugar maple leaves at 5 and 10°C, which yielded a rate increase of 0.0834 mg leaf material·mg biomass^{-1}·°C^{-1}. Consumption rates also increase with increased conditioning time. We determined the change in slope and *y*-intercept in the relationship between *Pycnopsyche* sugar maple consumption rates and biomass as

$$\text{Slope} = \text{Initial slope} + (0.0064 \times \text{days conditioned})$$

$$y\text{-intercept} = \text{Initial } y\text{-intercept} + (0.0220 \times \text{days conditioned}).$$

This was applied to all leaf species.

We had less data on consumption rates for *Peltoperla* than for *Pycnopsyche*. Consumption rates as a function of processing category are shown in Table 3 and reflect the same processing categories as for *Pycnopsyche*. We did not have sufficient data to develop the same kind of relationships between biomass and consumption rate for *Peltoperla*; therefore, *Peltoperla* rates do not change as the larvae grow. We used the same increase in consumption rate due to temperature determined for *Pycnopsyche* (0.0834 mg leaf material·mg biomass^{-1}·°C^{-1}) for *Peltoperla*.

We determined the change in consumption rates with increased conditioning by determining the difference in the consumption of sugar maple leaves conditioned for 8 and 12 weeks and assuming that the increase was linear. The rate increase of 0.0056 mg consumed·mg biomass^{-1}·day conditioned^{-1} was applied to fast- and medium-processed leaves. The increase in *Peltoperla* consumption rate of slow-processed leaves due to conditioning (0.0036 mg consumed·mg biomass^{-1}·day conditioned^{-1}) was determined by dividing the consumption rate of red oak by the number of days that it was conditioned.

We had no information regarding *Leuctra* feeding rates. The consumption rates determined for *Peltoperla* were used to simulate *Leuctra* feeding rates because *Peltoperla* was the closest in size and taxonomic relationship to *Leuctra*. Literature values were used for *Tipula* and *Cambarus*. We used the feeding rates reported by Vannote and Sweeney (1985) for *T. abdominalis*. Consumption rates were adjusted for temperature as described for *Peltoperla*. Feeding rates computed for *Tipula* were not adjusted for changing food quality over time due to lack of data. We used Webster's (1983) estimate of 0.031 mg dry weight consumed·mg biomass^{-1}·day^{-1} for the feeding rate of *Cambarus*. This rate was not adjusted for either stream temperature or changing food quality over time.

At each 1-m interval along Wilson Hollow, the model determined which of the available leaves were the most palatable based on processing category and degree of conditioning (Cummins and Klug 1979). The model then determined the "demand," i.e., the quantity that the invertebrates would consume based on invertebrate biomass, the quality of leaves available, and water temperature. If

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Table 2. Relationships between consumption and biomass for *Pycnopsyche* sp.

Experimental conditions	Regression equation	r^2
Sugar maple (2 weeks, 10°C)	Consumption = 0.404(ln weight) + 1.311	0.75
Sugar maple (8 weeks, 10°C)	Consumption = 0.674(ln weight) + 2.264	0.92
Sugar maple (12 weeks, 10°C)	Consumption = 0.673(ln weight) + 2.485	0.88
Sugar maple (12 weeks, 5°C)	Consumption = 0.256(ln weight) + 1.170	0.43
Red oak (13 weeks, 10°C)	Consumption = 0.582(ln weight) + 1.900	0.90
Dogwood (4 weeks, 10°C)	Consumption = 0.782(ln weight) + 2.465	0.85

Note: Leaf species, with conditioning time and the temperature at which consumption rates were measured, regression equations, and corresponding r^2 values are given.

Table 3. *Peltoperla* consumption rates (mg dry weight leaf·mg dry weight insect^{-1}·day^{-1}).

Leaf species	Consumption rate	*n*
Dogwood (4 weeks)	0.570	19
Sugar maple (8 weeks)	0.432	3
Sugar maple (12 weeks)	0.589	2
Red oak (13 weeks)	0.327	2

Note: Conditioning time of leaf material is given in parentheses. All consumption rates were measured at 10°C.

there were insufficient leaves of that category (a combination of leaf species and conditioning time), the model would then determine the next most palatable substrate until either it ran out of leaves or the demand was satisfied. In the model, invertebrate consumption was not allowed when discharge fell below 0.76 cm^3·s^{-1}, which approximates the discharge necessary to wet the rocks on the stream bottom.

Results

Leaves were processed in the model in much the same manner as has been observed in many studies of coarse particulate organic matter (CPOM) dynamics in streams (Fig. 3). Palatable leaves such as basswood and white ash were immediately consumed by invertebrates with little material processed by microbes and insignificant amounts lost to export. Less palatable leaves (tulip poplar, black birch, American hornbeam, and sugar maple) were initially processed by microbes and then consumed by invertebrates later in the winter. A small fraction was exported during storm events. Unpalatable leaves (American beech and red oak) were almost exclusively processed by microbes, with small amounts consumed by invertebrates in the spring and a small fraction exported. Approximately 44% remained at the end of the simulation.

Stream temperature was the major factor controlling processing rates. Leaves were processed quickly in the fall when temperatures were high; processing rates fell in winter due to the low temperatures and increased in the spring when water temperatures increased.

Under standard conditions, invertebrates consumed 30% of total leaf inputs, microbes processed 39% of inputs, 2% was exported, and 18% remained in the stream channel. The fates of leaves in the three processing categories varied. Invertebrates consumed 71% of fast-processed leaves as contrasted with 38% of medium-processed leaves and 4% of slow-processed leaves. Microbes processed 16% of fast-processed leaves, 41% of medium-processed leaves, and 45% of slow-processed leaves. Exports were 2% or less for all processing categories. CPOM left at the end of the simulation varied from 1% of fast-processed leaves to 2% of medium-processes leaves to 44% of slow-processed leaves.

Pycnopsyche was responsible for processing the majority of the invertebrate-processed inputs (52%) despite contributing 16.7% of annual mean shredder production and just 3% of annual mean biomass. *Peltoperla* and *Tipula* were also responsible for processing a major component of the leaf inputs, consuming 19 and 21%, respectively. *Leuctra* and *Cambarus* each processed just 4% of the inputs processed by invertebrates. The disparity between taxa in the level of processing was due to differences in feeding rates and biomass.

Sensitivity analysis

Leaf litter inputs vary from year to year due to environmental variables such as drought and terrestrial herbivory. To determine how sensitive the model was to variation in leaf litter inputs, we ran simulations where we increased or decreased inputs by 10%, which approximates the normal variation in litter inputs observed in the FEF (Fig. 4). The processing of slow-processed leaves was unchanged during all simulations. Invertebrates consumed a slightly smaller percentage of fast- and medium-processed leaves when inputs were increased and a slightly larger percentage when inputs were decreased. This was balanced by minor fluctuations in microbial processing, and for medium-processed leaves, minor shifts in CPOM remaining at the end of the simulation. In the model, invertebrate growth and survival rates were not adjusted for food availability, which probably contributed to the lack of sensitivity to changing leaf inputs.

Invertebrate densities are extremely variable in space and time (Downing 1979; Paller 1996). We evaluated the sensitivity of the model to variation in invertebrate densities. Invertebrate densities were increased or decreased 20% from normal conditions. Processing of slow-processed leaves was unaffected (Fig. 4). As invertebrate density increased, a greater percentage of fast- and medium-processed leaves was consumed by invertebrates. In fast-processed leaves, this was balanced by smaller percentages being processed by microbes. There was a slight reduction in the percentage of microbial processing of medium-processed leaves and a 10% reduction in CPOM remaining at the end of the simulation.

The model was robust with respect to transport and microbial processing rates. A 10% increase or decrease in the transport rate resulted in a less than 1% change in exports from all processing groups. A 10% increase or decrease in microbial processing rate also resulted in minimal changes from the normal simulation. A 50% increase in microbial processing rate increased the percentage of leaves processed

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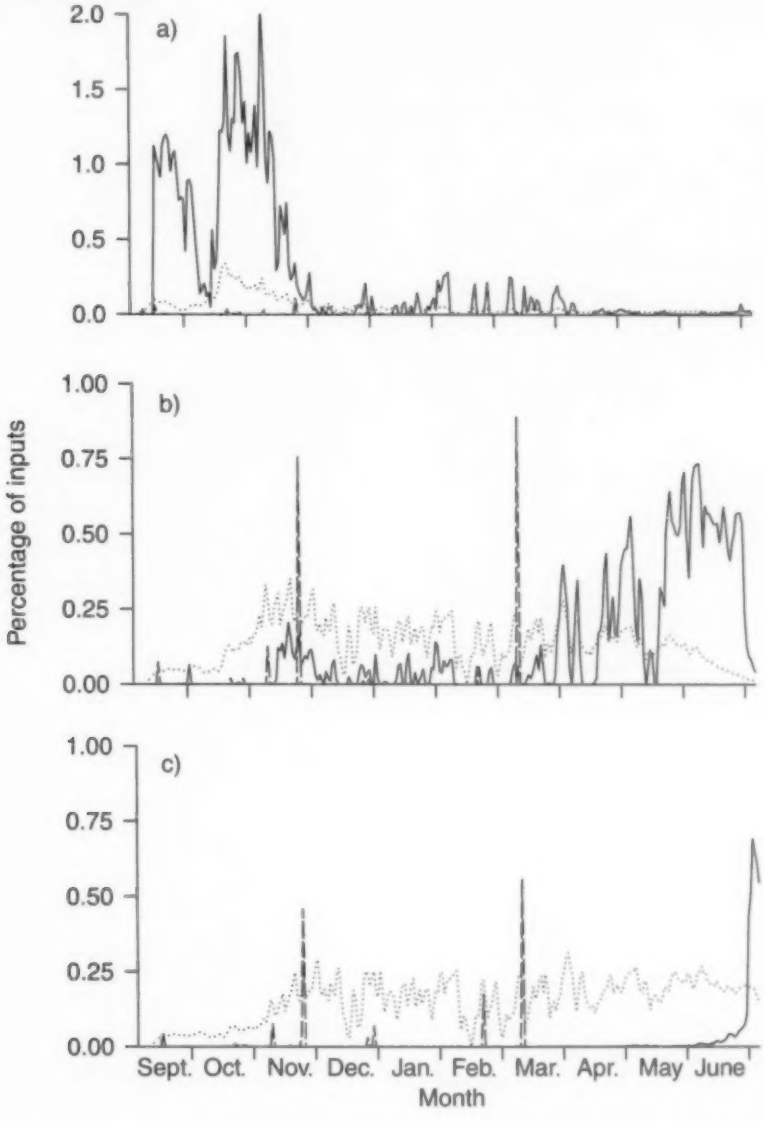
Fig. 3. Simulated processing dynamics of (*a*) fast-processed, (*b*) medium-processed, and (*c*) slow-processed leaves. Values are the percentage of inputs processed daily by invertebrates (solid line) and microbes (dotted line) or exported (dashed line) from the system. Note the changes in the *y*-axis in the graphs.



by microbes from 30 to 39%, mostly at the expense of material remaining at the end of the simulation. Leaves in the medium- and slow-processing groups were more affected than leaves in the fast-processing group.

These simulations demonstrated that, while output from the model is affected by changes in leaf litter inputs, transport, and microbial processing rates or invertebrate densities, the overall pattern of processing remained unchanged. The magnitude of the changes in model output were relatively small.

Climate change

Precise predictions regarding the effects of climate change on a specific region are difficult to make because the general circulation models simulate on a global scale rather than on a regional scale (Schneider 1993). General predictions regarding the FEF include warmer winter temperatures, increased winter precipitation, and possibly drier conditions overall due to increased evapotranspiration (Schneider et al. 1990; Moore et al. 1997). We ran four simulations designed to cover some of the possible outcomes. We selected discharge records from a wet and a dry year. After we ran simulations with those discharge records, we increased the stream temperature by 2°C and reran the simulations (Fig. 5). It is expected that the region will experience an average annual increase in air temperature of 3–4°C with a

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Fig. 4. Sensitivity analysis. In both graphs, the bars represent normal simulation results. F, M, and S correspond to the fast-, medium-, and slow-processing categories, respectively. (*a*) Effects of a 10% increase or decrease in leaf inputs. – – –, simulation where inputs were decreased; + + +, simulation where inputs were increased. (*b*) Effects of a 20% increase or decrease in invertebrate density. – – –, simulation where densities were decreased; + + +, simulation where densities were increased.

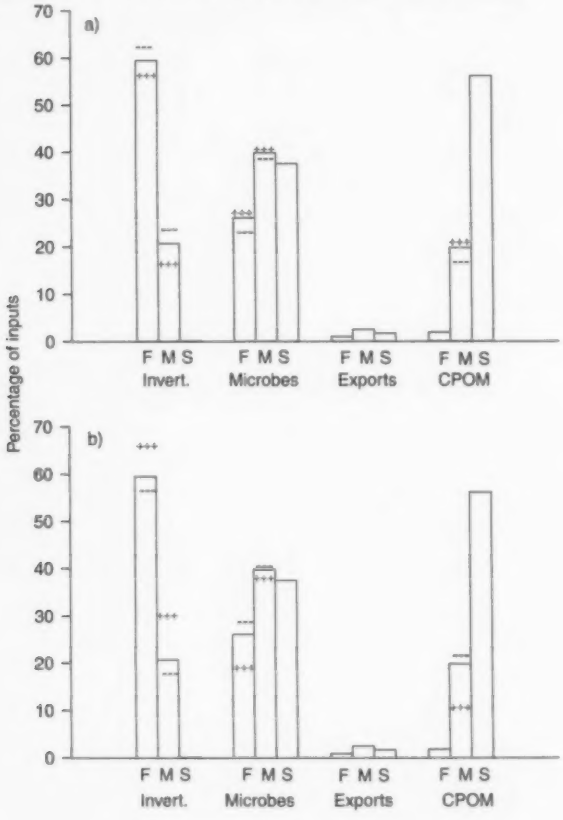
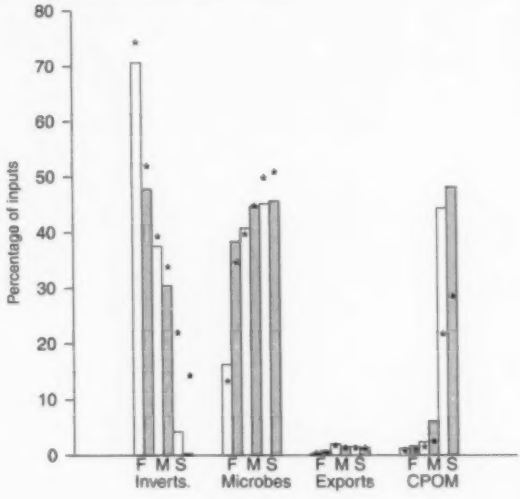


Fig. 5. Impacts of elevated water temperatures on processing dynamics during wet and dry years. Shown is the percentage of inputs processed by invertebrates and microbes or exported and remaining at the end of the simulations during a wet year (open bars) and a dry year (shaded bars) with F, M, and S corresponding to fast-, medium-, and slow-processing categories, respectively. Values for each category under the elevated temperature scenario are represented by an asterisk.



doubling in atmospheric CO_2 concentration (Moore et al. 1997); our regression equation predicts that this will correspond to a 2°C increase in stream temperature.

Invertebrates consumed a greater percentage of leaf inputs in wet years than in dry years (Fig. 5). Presumably, this is because during dry years, the stream channel remained dry longer in autumn, reducing invertebrate access to detrital material until discharge increased. Invertebrate consumption was at the expense of microbial processing for fast-processed leaves and at the expense of CPOM remaining at the end of the simulation for medium-processed leaves.

In scenarios where stream temperature was increased, invertebrates consumed more leaf material regardless of processing category. This increase may be underestimated by the model because increases in palatability due to conditioning are not calculated on a degree-day basis and therefore do not incorporate possible temperature effects. The increase in invertebrate consumption of fast-processed leaves was at the expense of microbial processing, with little change in the amount exported or remaining at the end of the simulation. In medium-processed leaves, invertebrates consumed more leaf material and microbes processed a slightly greater percentage. Both these activities were at the expense of CPOM remaining at the end of the simulation. A slight increase in the total amount of material processed by invertebrates and microbes created a decrease in CPOM remaining at the end of the simulation.

Climate change is predicted to increase the magnitude and frequency of high-flow events. This prediction could result in a wide variety of scenarios. We addressed two possibilities. We ran a simulation, which increased the magnitude of storm events already present in the discharge record by 10%. This had little effect; exports increased less than 1%.

We also examined the impact of a large storm event on leaf processing. We ran simulations with a 20-year flood event occurring in November during the leaf fall period, in January after leaf fall but while leaves still were being blown into the stream, and in March after the majority of leaf processing had occurred. Storms later in the year exported slightly less material; however, the timing of the storm event had little effect on the fate of the leaf material. The impact of the storm, regardless of when it occurred, was to export substantial amounts of medium- and slow-processed leaves and lesser amounts of fast-processed leaves (Fig. 6). These losses were reflected in the lower percentages of leaves processed by invertebrates and microbes and significant reductions in the amount of CPOM remaining at the end of the simulation.

A final simulation was run combining the effects of a large storm event and elevated stream temperatures. Invertebrate consumption of fast-processed material increased to

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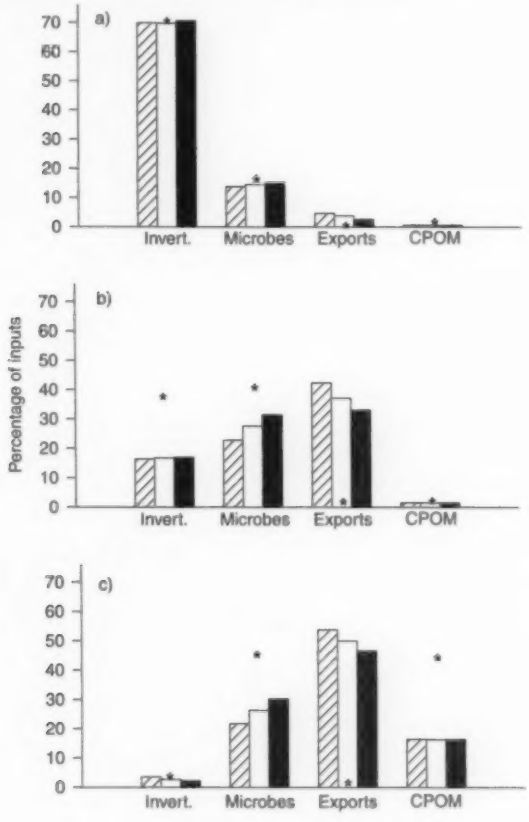
Fig. 6. Impacts of a 20-year storm occurring in November (hatched bars), January (open bars), or March (solid bars) on (*a*) fast-processed, (*b*) medium-processed, and (*c*) slow-processed leaves. The asterisk indicates standard conditions.



75.4% with reductions in microbial processing and exports. Processing of medium-processed leaves by both invertebrates and microbes increased to 20.4 and 29.1%, respectively. Exports of medium-processed leaves decreased to 32.3%, and only 0.9% was left at the end of the simulation. Invertebrates consumed 8.5% of slow-processed leaves, with 30% processed by microbes and 48% exported. Only 9% remained at the end of the simulation.

Discussion

The model reproduced the processing continuum, with fast-processed leaves being consumed by invertebrates, allowing less palatable leaves to be conditioned by microbes before invertebrates consume them. Simulation results were in good agreement with most of the field and modeling studies that partitioned mass loss to invertebrates, microbes, or exports (Table 4). Studies that failed to differentiate between throughput (inputs from upstream sources that are passed through the study area) and export of inputs to the reach have not been included in the comparison. In most studies, invertebrates consumed between 13 and 20% of leaf inputs, microbes processed 43 to 48% of inputs, and, except for the study by Fisher and Likens (1973), exports ranged between 0.8 and 4.2%.

Table 4. Comparison of literature and simulation results.

Study	Invertebrates	Microbes	Exports
Cummins et al. 1973	20		
Fisher and Likens 1973	18[a]		30
Hall 1972			~2
McDowell and Fisher 1976			3.6
Petersen et al. 1989	18.6[b]	48.1	
Wallace et al. 1982	14		
Webster 1983	13	43.2	0.8
Webster and Patten 1979	80		4.2
This study	30.0	38.9	1.6

Note: Values are expressed as percentages of total leaf inputs.
[a]Recalculated by Webster (1983).
[b]Recalculated using a 20% assimilation efficiency to convert assimilation to ingestion.

Simulation results predicted that, in general, higher stream temperatures would have greater impacts on leaf pack processing than would be expected by variations in leaf inputs or invertebrate densities. Higher stream temperatures led to substantial increases in invertebrate consumption and microbial processing during wet and dry years. Simulation results also indicated that the invertebrate response to elevated temperatures was stronger than the microbial response, with the invertebrates processing more of the available inputs than the microbes. Increased invertebrate processing will lead to a greater percentage of leaf inputs being converted to FPOM. This may lead to increased collector densities, although the strength of the connection between shredding activity and collector densities has been questioned by Heard and Richardson (1995). Moreover, the data regarding microbial processing rates with respect to temperature are very scant. If the microbial response to temperature is actually exponential, rather than linear as we represented it in the model, microbial processing would be increased in the elevated temperature scenarios and possibly under standard conditions. This reduces the leaf biomass available to shredders as well as collector–gatherers that consume the leaf fragments created by the shredders.

Increased processing during winter months substantially reduces CPOM stocks in the summer months. In addition, the remaining detrital material consists of the lower quality, more slowly processed red oak and American beech leaves. Under these conditions, competition for detrital resources will be higher, leading to decreases in growth, fecundity, and production of shredding macroinvertebrates. Richardson (1991) demonstrated that under food-limiting conditions, shredding invertebrates had lower densities and mass.

Another component of climate change, the increase in the frequency and magnitude of high-flow events, may have more impact on leaf pack processing. Regardless of when they occur, large storm events export substantial quantities of detrital material. Increased winter processing rates in conjunction with a very large storm reduced total CPOM stocks to just 2.4% of the inputs. Unless shredding invertebrates can use alternative food resources during summer, it is hard to envision how they will be maintained in the headwater stream community.

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Sweeney et al. (1991) suggested that changes in the timing of leaf inputs might occur with climate change. This is unlikely to be an important factor within our study region, as the initiation of leaf abscission in most of the major taxa in these forests is controlled either by photoperiod or by a combination of photoperiod and temperature, with photoperiod being the more important factor (Kramer 1936). Under climate change scenarios, with elevated temperatures and reduced precipitation during the summer, trees may experience more drought stress, causing premature leaf fall (Kozlowski 1991). In this case, however, the leaves would most likely fall on a dry streambed and be available when discharge increased later in the year.

The ability of the model to accurately predict the effects of climate change on leaf pack processing is limited by a lack of information regarding the response of macroinvertebrates to elevated temperatures. In experiments where stream channels were maintained at elevated temperatures, the macroinvertebrate response was taxon specific and ranged from normal developmental rates and smaller adults to accelerated development and earlier maturation (Rempel and Carter 1987; Hogg and Williams 1996). The response of each taxon may have considerable influence on leaf pack processing dynamics, as there were considerable differences in consumption between taxa.

While the developmental response of most aquatic invertebrates to elevated temperatures is unknown, under all climate change scenarios, the quantity of CPOM remaining at the end of the spring is substantially reduced. This is likely to be further exacerbated by the increased probability of high-flow events exporting material downstream. It appears likely that those taxa that can accelerate their development will fare better than those that cannot.

Simulation results suggest that changes in canopy composition, such as suggested by Meyer and Pulliam (1991), may have significant impacts on the shredder community. Under all climate change scenarios, fast-processed material was generally consumed by macroinvertebrates before much microbial processing could occur. A greater percentage of medium-processed material was processed by both invertebrates and microbes, reducing CPOM stocks available through the summer. A shift in the canopy towards fast-processed leaves could further reduce the CPOM stocks available in the summer. Conversely, shifts in the canopy towards slow-processed leaves might increase the relative proportion of low-quality food during the winter, the major growing season, and increase CPOM stocks in the summer. Changes in food quality may have dramatic effects on growth and survival of aquatic invertebrates (Sweeney et al. 1991).

Changes in the processing of detrital material in the headwaters also impact downstream communities that are dependent on transport of FPOM to them. Under the elevated temperature scenarios, CPOM is processed earlier in the year, which would lead to increased transport of FPOM during the winter months. The earlier availability of FPOM will be at the cost of lowered FPOM availability later in the year.

In summary, our simulation results indicate that climate change will change leaf pack processing dynamics by increasing processing rates during the winter months. Shredding macroinvertebrates that can accelerate their development will likely do well under these conditions; however, those with long life histories probably will not. If the predicted increase in frequency and magnitude of storm events does occur, CPOM stocks will be further reduced, creating greater competition among shredding invertebrates. Any changes in processing dynamics in headwater streams, whether they are due to earlier processing of autumnal leaf inputs, massive exports due to floods, or other unidentified responses to climate change, will likely have impacts downstream due to temporal changes in FPOM availability.

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Application of statistical model selection criteria to the Stock Synthesis assessment program

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Abstract: Statistical modeling involves building sufficiently complex models to represent the system being investigated. Overly complex models lead to imprecise parameter estimates, increase the subjective role of the modeler, and can distort the perceived characteristics of the system under investigation. One approach for controlling the tendency to increased complexity and subjectivity is to use model selection criteria that account for these factors. The effectiveness of two selection criteria was tested in an application with the stock assessment program known as Stock Synthesis. This program, which is often used on the U.S. west coast to assess the status of exploited marine fish stocks, can handle multiple data sets and mimic highly complex population dynamics. The Akaike information criterion and Schwarz's Bayesian information criterion are criteria that satisfy the fundamental principles of model selection: goodness-of-fit, parsimony, and objectivity. Their ability to select the correct model form and produce accurate estimates was evaluated in Monte Carlo experiments with the Stock Synthesis program. In general, the Akaike information criterion and the Bayesian information criterion had similar performance in selecting the correct model, and they produced comparable levels of accuracy in their estimates of ending stock biomass.

Résumé : La modélisation statistique demande la construction de modèles suffisamment complexes pour représenter le système à l'étude. Si les modèles sont trop complexes, les estimations des paramètres manquent de précision, l'influence subjective du modélisateur est accrue et peut distordre les caractéristiques perçues du système à l'étude. Pour limiter la tendance à l'accroissement de la complexité et de la subjectivité, il est possible d'avoir recours à des critères de sélection du modèle qui tiennent compte de ces facteurs. Nous avons testé l'efficacité de deux critères de sélection dans une application à un programme d'évaluation des stocks (Stock Synthesis), souvent utilisé sur la côte ouest des États-Unis pour évaluer l'état des stocks de poissons marins exploités, qui peut manipuler des séries multiples de données et simuler une dynamique de population extrêmement complexe. Le critère d'information d'Akaike et le critère d'information bayésien de Schwarz sont des critères qui respectent les principes fondamentaux du choix d'un modèle : bon ajustement, parcimonie et objectivité. Nous avons évalué leur aptitude à choisir la forme correcte de modèle et à produire des estimations exactes en menant des expériences par la méthode de Monte Carlo sur le programme Stock Synthesis. En général, la performance du critère d'information d'Akaike et du critère d'information bayésien étaient similaires pour ce qui est du choix du modèle correct, et les modèles produisaient des niveaux comparables d'exactitude dans leurs estimations de la biomasse finale du stock.

[Traduit par la Rédaction]

Introduction

The Stock Synthesis program (Methot 1990) adapts the methods of Fournier and Archibald (1982) and Deriso et al. (1985) to reconstruct the demographic history of a fish stock from observed changes in age distributions of the catch coupled with auxiliary information such as survey indices of stock abundance. A major strength of the Stock Synthesis program is its ability to handle large amounts and different kinds of data. The program is very flexible with respect to the underlying population dynamics models and to the number of parameters that it can estimate.

The Stock Synthesis program (Appendix A) is structured to simultaneously analyze time series data on catch biomass, age (or size) composition, effort from multiple fisheries, and abundance and age composition data from multiple surveys. This program can accommodate ageing precision, ageing bias, and conversion of age to length by incorporating transition matrices that generate the expected distribution of the observed age compositions given the model's estimate of the true age compositions. The deviations between observations and the model's expected values are measured in terms of log-likelihood. The Stock Synthesis program does not include any formal mechanism for selecting an appropriate model structure, however. Choice of an appropriate structure is left to the user.

The model selection process, in the way the Stock Synthesis program is usually applied, overlooks two fundamental concerns in modeling: the process is overly subjective and it does not account for model complexity. Consequently, the chosen model can easily be overparameterized (Ianelli and Ito 1991), in which case the model has more parameters than the data can support, and comparable goodness-of-fit as attained by the chosen model can be achieved with a simpler alternative. Redundant parameters use up degrees of freedom

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and reduce the precision of the estimated parameters. In current practice, there is considerable subjectivity in setting up the Stock Synthesis program for the analysis of a particular data set. The application of the Stock Synthesis program would be greatly facilitated by having available objective techniques for judging between alternative model formulations.

In this study, we evaluate whether applications of the Stock Synthesis program can be improved by incorporating statistical model selection criteria that are objective and that account for model complexity. The Akaike information criterion (AIC) (Akaike 1973) and Schwarz's Bayesian information criterion (BIC) (Schwarz 1978) are two model selection criteria that have gained support in the recent literature (Forster and Sober 1994; Kass and Raftery 1995; Raftery et al. 1995). AIC and BIC both have desirable features: they measure goodness-of-fit but penalize model complexity and they are fully objective. In this study, we use Monte Carlo simulation experiments to generate random data sets with known characteristics. We then apply AIC and BIC to the process of building a stock assessment model with the Stock Synthesis program.

Materials and methods

Statistical model selection

Statistical model selection is a process designed to identify the optimal model from a set of competing models. Many frequently used criteria for model selection, including variations of the coefficient of determination (R^2), are based solely on their effectiveness at fitting the observed data and do not take into account model complexity. The F test and the likelihood ratio test account for model complexity, but both tests can only be applied to nested models, in which simpler model forms are obtained by equating or dropping parameters from more complex alternative forms. The Cp statistic (Mallows 1973) accounts for model complexity but only applies when the fitting method is least squares. The AIC and BIC, which have their roots in the method of maximum likelihood, account for model complexity and are easy to apply to nonnested models. Burnham and Anderson (1998) provide a thorough but very readable description of AIC and related model selection criteria.

Both AIC and BIC use maximum likelihood values to evaluate alternative model forms. If individual observations Y_i ($i = 1, 2, \ldots, n$) are randomly sampled from a population where the probability density function is $g(Y, \theta)$, then the likelihood function is

$$(1) \qquad \prod_{i=1}^{n} g(Y_i, \theta).$$

Those parameter values θ that maximize the likelihood (or log-likelihood) function are the maximum likelihood estimates.

The AIC and BIC are usually defined as

$$(2) \qquad AIC = -2\ln(L_{\max}) + 2p$$

$$(3) \qquad BIC = -2\ln(L_{\max}) + p\ln(n)$$

where $L_{\max}$ is the natural logarithm of the likelihood function obtained at the maximum likelihood estimates, p is the number of estimated parameters, and n is the observed number of data points. For model selection using either AIC or BIC, one seeks the model that minimizes the criterion. The BIC values obtained from different models can be used as approximate weights for estimating posterior probabilities for the different models in a Bayesian framework (Ramsey and Schafer 1997). Both AIC and BIC measure goodness-of-fit in terms of log-likelihood and penalize models with large numbers of parameters. However, they differ in their penalty functions, $2p$ versus $p\ln(n)$. Because $\ln(n) > 2$ for $n \geq 8$ and because models are usually applied to data sets with more than eight observations, in most applications, BIC imposes a harsher penalty for complexity than does AIC.

In our application of AIC and BIC with the Stock Synthesis program (Appendix B), we counted the observed number of data points, n, as the number of observed survey index values plus the number of observed fishing effort values plus the number of age-classes in the observed survey and fishery age composition data sets. The number of estimated parameters, p, included the fishery and survey selectivity function parameters, the fishery catchability coefficient, and initial age composition and recruitment parameters. The annual catch biomass data were not counted as part of n and the annual fishing mortality coefficients were not counted as part of p because the Stock Synthesis program assumes that the data for catch biomass are measured with perfect accuracy and adjusts the estimates for $F(y)$ so that each annual estimate of total catch biomass exactly matches the observed total catch biomass.

Experiments

The Stock Synthesis program can be configured to allow a wide variety of simplifications that reduce the number of unknown parameters. Three sets of configurations were investigated in our study. In the first experiment, the initial age composition of the stock was assumed to be in equilibrium with some constant level of historic recruitment. This was contrasted with a more complex model in which the stock was not initially at equilibrium because of variable historic recruitment (Fig. 1). In both cases, the historic rates of natural mortality were constant, the historic rates of fishing mortality were zero, and fishery selectivity was domed. The simpler model (equilibrium age composition) is nested within the more complex model because the simpler version is obtained by setting the historic recruitment to a single value. The simpler model has $A - 1$ fewer parameters than the nonequilibrium alternative, where A is the number of distinct age-classes in the age composition data.

The second experiment contrasted age-dependent natural mortality with domed fishery selectivity. In the first case, the stock was assumed to experience age-dependent rates of instantaneous natural mortality coupled with an asymptotic fishery selectivity function (Fig. 2). This was contrasted with a stock that had a constant rate of natural mortality coupled with a domed selectivity function. Both scenarios attempt to account for a relative absence of older fish in the catch. Both were proposed as explanations for the lack of older females in recent assessments of the stock of canary rockfish off Oregon and Washington (Sampson 1996). The first scenario has one parameter to set the level of natural mortality at the terminal age with a linear trend from a known initial level, starting at ages greater than a known transition age. The second has a known constant rate of natural mortality but two unknown parameters for the inflection age and the slope of the descending portion of the selectivity curve. The first scenario has one less free parameter than the alternative but is not nested within it.

The third experiment contrasted asymptotic fishery selectivity with domed fishery selectivity. Both scenarios had the same constant natural mortality rate for all ages. The first scenario is nested within the second and has two less free parameters.

Random data sets were generated for the three experiments using two C++ programs. The underlying equations of the Stock Synthesis program (Appendix A) were reproduced in the first program to simulate the dynamics of an age-structured population. The second program produced random replicate data sets corresponding to the population generated by the first program (Appendix B). The random data sets were then analyzed by the Stock Synthesis program, first assuming the correctly specified model structure and then assuming the incorrect alternative.

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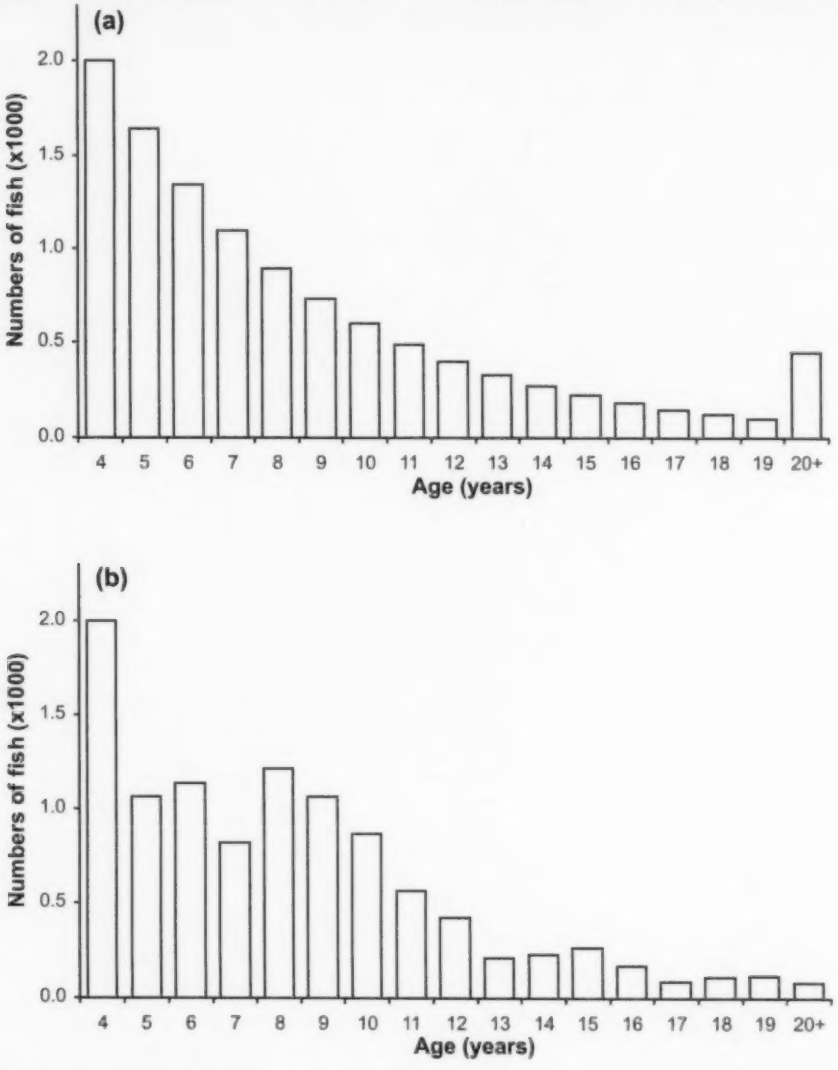
Fig. 1. Alternative scenarios of the first experiment: (*a*) equilibrium versus (*b*) nonequilibrium initial age composition.



We applied the AIC and BIC model selection criteria to the log-likelihood values output by the Stock Synthesis program and determined whether these criteria led to choosing the correct model or the incorrect counterpart. Because the correct model specification may not produce the most accurate model predictions, we also calculated the average squared deviations of the Stock Synthesis estimates of ending stock biomass from the true values. The estimates of ending biomass are often of great interest to fishery managers because these estimates largely determine the magnitude of the next harvest quota.

We use the term "correct" to describe the model that we originally used to generate the random data and "incorrect" for the alternative. With nested models, as in experiments 1 and 3, when the true scenario is the simpler one, it would be more accurate to describe the more complex alternative as being overparameterized rather than incorrect. When the true scenario is the more complex one, however, the simpler alternative is wrong.

Experimental design

In applications with the Stock Synthesis program, the effectiveness of a given model selection criterion may depend on the demographic characteristics of the stock. Our experiments were designed to examine the effects of four factors on the performance of the model selection criteria. To keep the experiments simple, only two levels (low and high) of each of the four factors (or 2^4 = 16 treatment combinations) were examined. The four factors (and their low and high levels) were (*i*) data variability, which included the size of the annual age composition samples (400 versus 100 fish·year^{-1}), the coefficient of variation of the annual fishing effort data (20 versus 80%, arithmetic scale), and the coefficient of variation of the annual survey biomass estimates (20 versus 80%, arithmetic scale), (*ii*) recruitment variability, which included three recruitment sequences (constant recruitment versus a low variability sequence and the low variability sequence versus a high variability sequence), all with the same mean (2000), (*iii*) fishing

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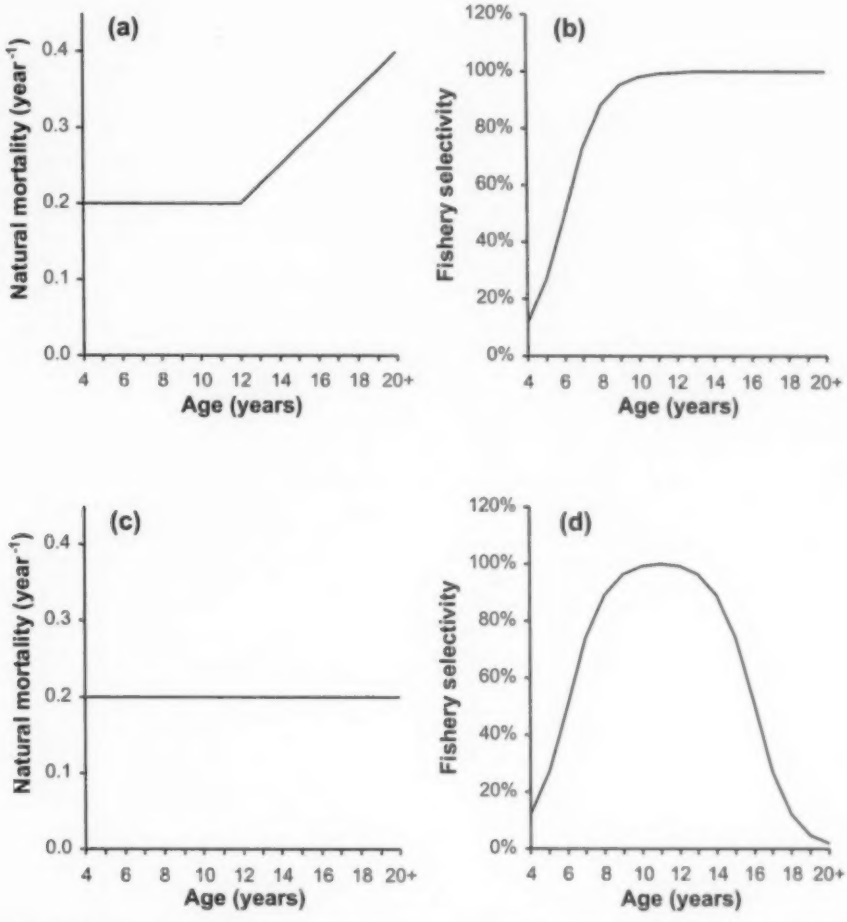
Fig. 2. Alternative scenarios of the second experiment: (*a*) age-dependent natural mortality with (*b*) an asymptotic fishery selectivity function versus (*c*) constant natural mortality with (*d*) a domed fishery selectivity function. The alternative scenarios of the third experiment were asymptotic fishery selectivity (Fig. 2*b*) versus domed fishery selectivity (Fig. 2*d*), both scenarios having constant natural mortality.



mortality trend, which was the annual increase in the rate of fishing mortality (0.01 versus 0.03·year^{-1}), and (*iv*) natural mortality (0.2 versus 0.4·year^{-1}) and the range of age-classes in the data series (2–9 plus an accumulator class for M = 0.4·year^{-1} versus 4–19 plus an accumulator class for M = 0.2·year^{-1}).

In all scenarios, except for the first experiment's nonequilibrium alternative, the initial age compositions were in equilibrium with a constant level of historic recruitment (2000 fish annually), and the two levels of recruitment variability consisted of continued constant recruitment during the simulated period or a sequence of annual recruitment values having low variability (2000, 2300, 1800, 1200, 1900, 2400, 1700, 1300, 2100, 2300, 2900, 2900, 2700, 1500, 1700, and 1300). Because constant recruitment would have resulted in an equilibrium initial age composition, for the nonequilibrium scenario of experiment 1, the initial age composition was obtained from a sequence of variable annual recruitment values during the years preceding the simulated period. The same variable sequence was also used for the simulated period. The low variability sequence was identical to the variable sequence used in experiments 2 and 3 and for the equilibrium scenario of experiment 1. The high variability sequence was 2000, 600, 900, 3600, 700, 3200, 2600, 1200, 800, 3900, 2100, 1000, 2900, 900, 3000, and 2600.

When M was 0.2·year^{-1}, the ascending and descending inflection ages for the fishery selectivity function were 6 and 16 years and the ascending inflection age for the survey selectivity function was 5 years. When M was 0.4·year^{-1}, the ascending and descending inflection ages for the fishery selectivity function were 4 and 8 years and the ascending inflection age for the survey selectivity function was 3 years. The ascending slope coefficients for the fishery and survey selectivity functions for all simulated stocks were 1.0 and 1.5·year^{-1}, respectively. When the fishery selectivity function was domed, the descending slope coefficient was 1.0·year^{-1}.

The second experiment, which included an age-dependent function for natural mortality, required a minimum M, a maximum M, and a transition age where M began changing linearly. At the low natural mortality level, the parameters were set at M_{min} = 0.2·year^{-1}, M_{max} = 0.4·year^{-1}, and a transition age of 12 years. At the high natural mortality level, the parameters were set at M_{min} = 0.4·year^{-1}, M_{max} = 0.6·year^{-1}, and a transition age of 6 years.

For each experiment, we created a population for each treatment combination and each of the two contrasting scenarios, 2 × 16 = 32

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Table 1. Experiment 1: equilibrium versus nonequilibrium initial age composition.

Stock	Experimental treatments: Data variability	Recruitment variability	F trend	M	Correct selections: AIC	BIC	MSRE (%): AIC	BIC	Correct	Incorrect	True ending biomass
Equilibrium (true) versus nonequilibrium (alternative) initial age composition											
1	Low	None	0.01	Low	100	100	1.32	1.32	1.32	1.65	30 994.1
2	Low	None	0.01	High	98	100	2.21	2.21	2.21	2.12	7 455.3
3	Low	None	0.03	Low	100	100	1.26	1.26	1.26	1.37	21 234.0
4	Low	None	0.03	High	96	100	1.48	1.46	1.46	1.52	5 312.0
5	Low	Low	0.01	Low	98	100	1.64	1.57	1.57	1.83	32 003.9
6	Low	Low	0.01	High	99	100	2.33	2.32	2.32	2.71	7 787.4
7	Low	Low	0.03	Low	100	100	0.72	0.72	0.72	0.75	21 186.6
8	Low	Low	0.03	High	98	100	1.46	1.51	1.51	1.63	5 274.8
9	High	None	0.01	Low	99	100	2.55	2.56	2.56	2.84	30 994.1
10	High	None	0.01	High	98	100	6.72	6.70	6.70	6.39	7 455.3
11	High	None	0.03	Low	99	100	1.19	1.19	1.19	1.30	21 234.0
12	High	None	0.03	High	98	100	2.70	2.62	2.62	2.96	5 312.0
13	High	Low	0.01	Low	100	100	1.99	1.99	1.99	2.60	32 003.9
14	High	Low	0.01	High	100	100	8.00	8.00	8.00	8.26	7 787.4
15	High	Low	0.03	Low	99	100	1.53	1.53	1.53	1.58	21 186.6
16	High	Low	0.03	High	96	100	1.74	1.72	1.72	2.09	5 274.8
Nonequilibrium (true) versus equilibrium (alternative) initial age composition											
17	Low	Low	0.01	Low	100	100	2.65	2.65	2.65	4.37	31 845.0
18	Low	Low	0.01	High	100	100	2.58	2.58	2.58	10.60	7 783.0
19	Low	Low	0.03	Low	100	100	0.81	0.81	0.81	0.82	21 049.8
20	Low	Low	0.03	High	100	100	1.38	1.38	1.38	3.66	5 272.3
21	Low	High	0.01	Low	100	100	1.52	1.52	1.52	14.92	32 674.0
22	Low	High	0.01	High	100	100	2.19	2.19	2.19	42.06	7 849.8
23	Low	High	0.03	Low	100	100	1.29	1.29	1.29	5.41	22 770.5
24	Low	High	0.03	High	100	100	1.52	1.52	1.52	8.55	5 682.8
25	High	Low	0.01	Low	100	69	3.36	3.35	3.36	2.92	31 845.0
26	High	Low	0.01	High	100	51	6.34	8.12	6.34	10.16	7 783.0
27	High	Low	0.03	Low	100	64	1.75	1.71	1.75	1.66	21 049.8
28	High	Low	0.03	High	100	59	2.38	2.73	2.38	3.30	5 272.3
29	High	High	0.01	Low	100	100	2.90	2.90	2.90	9.07	32 674.0
30	High	High	0.01	High	100	100	7.09	7.09	7.09	59.03	7 849.8
31	High	High	0.03	Low	100	100	1.52	1.52	1.52	2.32	22 770.5
32	High	High	0.03	High	100	100	2.15	2.15	2.15	7.02	5 682.8

stocks. For each stock, we generated 100 random replicates for a total of 32 × 100 = 3200 experimental units in each experiment. With this level of replication, there was at least an 80% probability that the experiments would detect differences of 20% or greater in the ability of the criteria to choose the correct model. We analyzed each experimental unit twice using the Stock Synthesis program, once assuming the stock's correct structural specification and again assuming the incorrect alternative.

We applied the two model selection criterion (AIC and BIC) and recorded the number of times the correct model structure was chosen and the average squared relative deviation from the true ending biomass of the estimated ending biomass (the mean squared relative error, MSRE) for whichever model structure was chosen, averaged over the 100 replicates for each experimental treatment:

$$(4) \qquad MSRE = \frac{1}{100}\sum\left(\frac{estimated\ biomass}{true\ ending\ biomass} - 1\right)^2.$$

To standardize the MSRE measures of accuracy, we calculated an additional set of MSRE values based only on the correct model. Furthermore, to measure the potential range of MSRE values, we also calculated a set of MSRE values based only on the incorrect alternative.

For those portions of the experiment that produced variability in the results, we used analysis of variance (ANOVA) to evaluate the importance of the four experimental factors. We transformed the proportion of the replicates that resulted in selecting the correct model structure using the logit transformation (Ramsey and Schafer 1997) and then applied the ANOVA to the logit data:

$$(5) \qquad Y = \ln\left(\frac{R_i/n}{1 - R_i/n}\right)$$

where R_i is the number of times the correct model structure was chosen for experimental treatment i and n is the number of replicate trials (100 in all cases). The following transformations were used for the extreme values of R_i: $1/(2n)$ was substituted for $R_i = 0$ and $1 - 1/(2n)$ was substituted for $R_i = 100$. To examine the effects of the four experimental factors on the accuracy of the estimated ending biomass, we applied ANOVA to the logarithm of the ratio of the MSRE values from the selected models over the MSRE for the correct model.

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Table 2. Experiment 2: age-dependent natural mortality (with asymptotic fishery selectivity) versus domed fishery selectivity (with constant natural mortality).

	Experimental treatments				Correct selections		MSRE (%)				
Stock	Data variability	Recruitment variability	*F* trend	*M*	AIC	BIC	AIC	BIC	Correct	Incorrect	True ending biomass
Age-dependent *M* (true) versus domed fishery selectivity (alternative)											
33	Low	None	0.01	Low	100	100	1.84	1.84	1.84	3.91	28 690.3
34	Low	None	0.01	High	100	100	2.33	2.33	2.33	7.36	7 250.1
35	Low	None	0.03	Low	100	100	1.31	1.31	1.31	1.45	19 852.0
36	Low	None	0.03	High	100	100	1.18	1.18	1.18	1.70	5 489.5
37	Low	Low	0.01	Low	100	100	1.88	1.88	1.88	4.23	29 733.2
38	Low	Low	0.01	High	100	100	2.92	2.92	2.92	7.32	7 704.3
39	Low	Low	0.03	Low	100	100	1.24	1.24	1.24	1.38	19 831.0
40	Low	Low	0.03	High	100	100	1.49	1.49	1.49	1.82	5 578.9
41	High	None	0.01	Low	100	100	2.72	2.72	2.72	3.66	28 690.3
42	High	None	0.01	High	100	100	3.18	3.18	3.18	7.32	7 250.1
43	High	None	0.03	Low	100	100	1.91	1.91	1.91	1.91	19 852.0
44	High	None	0.03	High	94	98	2.52	2.47	2.50	2.46	5 489.5
45	High	Low	0.01	Low	100	100	2.40	2.40	2.40	2.31	29 733.2
46	High	Low	0.01	High	99	99	8.24	8.24	8.20	7.75	7 704.3
47	High	Low	0.03	Low	100	100	1.57	1.57	1.57	1.55	19 831.0
48	High	Low	0.03	High	100	100	1.87	1.87	1.87	2.39	5 578.9
Domed fishery selectivity (true) versus age-dependent *M* (alternative)											
49	Low	None	0.01	Low	100	100	1.48	1.48	1.48	1.97	30 994.1
50	Low	None	0.01	High	100	100	2.84	2.84	2.84	4.30	7 455.3
51	Low	None	0.03	Low	100	100	0.79	0.79	0.79	1.19	21 234.0
52	Low	None	0.03	High	100	100	1.29	1.29	1.29	1.31	5 312.0
53	Low	Low	0.01	Low	100	100	2.32	2.32	2.32	2.92	32 003.9
54	Low	Low	0.01	High	100	100	2.63	2.63	2.63	4.00	7 787.4
55	Low	Low	0.03	Low	100	100	0.79	0.79	0.79	1.18	21 186.6
56	Low	Low	0.03	High	100	100	1.63	1.63	1.63	1.64	5 274.8
57	High	None	0.01	Low	100	100	2.74	2.74	2.74	3.79	30 994.1
58	High	None	0.01	High	100	100	7.10	7.10	7.10	5.45	7 455.3
59	High	None	0.03	Low	100	100	1.55	1.55	1.55	1.53	21 234.0
60	High	None	0.03	High	100	100	2.10	2.10	2.10	1.92	5 312.0
61	High	Low	0.01	Low	100	100	1.74	1.74	1.74	2.21	32 003.9
62	High	Low	0.01	High	100	100	4.88	4.88	4.88	5.87	7 787.4
63	High	Low	0.03	Low	100	100	1.53	1.53	1.53	1.64	21 186.6
64	High	Low	0.03	High	100	100	2.06	2.06	2.06	2.09	5 274.8

For analyzing the logit values and logarithm of the MSRE ratios from the first experiment, we used an ANOVA model that included main effects and two-way interactions for the four experimental treatments. To increase the degrees of freedom for the error sum of squares, the three- and four-way interactions were assumed to be negligible and were left confounded with the residual error. For analyzing the results from the second and third experiments, we used the same approach but included a main effect for the model selection criterion (AIC versus BIC) and the corresponding two-way interactions. This extra effect was not required with the results from the first experiment because only one model selection criterion produced variable results.

Results

The AIC and BIC model selection criteria were very similar in performance over the range of experimental conditions examined and in most all cases resulted in selection of the correct model. In general, AIC and BIC produced comparable levels of accuracy in their estimates of ending biomass.

In the first experiment (Table 1), BIC always selected the correct model for the simpler scenario (equilibrium initial age composition), but AIC occasionally selected the incorrect alternative. In the ANOVA of the logit values from the AIC selections, only the natural mortality factor was significant ($P < 0.05$). The ending biomass estimates from the AIC selections were slightly less accurate than the estimates from the correct model. In the ANOVA of the logarithm of the MSRE ratios (the MSRE from the AIC selections over the MSRE from the correct model), no factors were significant ($P < 0.05$). With the more complex scenario (nonequilibrium initial age composition), AIC always selected the correct model, but BIC was markedly erroneous for the stocks that had high data variability and low recruitment variability (stocks 25–28). In the ANOVA of the logit values from the BIC selections, the factors data variability and recruitment

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Table 3. Experiment 3: asymptotic versus domed fishery selectivity.

Stock	Experimental treatments				Correct selections		MSRE (%)				True ending biomass
	Data variability	Recruitment variability	F trend	M	AIC	BIC	AIC	BIC	Correct	Incorrect	
Asymptotic (true) versus domed fishery selectivity (alternative)											
65	Low	None	0.01	Low	100	100	1.41	1.41	1.41	1.43	29 347.2
66	Low	None	0.01	High	98	100	2.52	2.49	2.49	2.49	7 505.2
67	Low	None	0.03	Low	97	100	1.45	1.46	1.46	1.44	19 940.8
68	Low	None	0.03	High	99	100	1.35	1.35	1.35	1.36	5 555.3
69	Low	Low	0.01	Low	98	100	1.77	1.76	1.76	1.78	30 376.1
70	Low	Low	0.01	High	100	100	2.29	2.29	2.29	2.39	7 940.4
71	Low	Low	0.03	Low	100	100	0.96	0.96	0.96	0.96	19 917.4
72	Low	Low	0.03	High	99	100	1.03	1.03	1.03	1.03	5 640.5
73	High	None	0.01	Low	98	100	1.70	1.69	1.69	1.73	29 347.2
74	High	None	0.01	High	97	100	3.43	3.29	3.29	3.57	7 505.2
75	High	None	0.03	Low	96	100	1.85	1.85	1.85	1.84	19 940.8
76	High	None	0.03	High	97	100	1.87	1.87	1.87	1.89	5 555.3
77	High	Low	0.01	Low	95	100	1.53	1.52	1.52	1.53	30 376.1
78	High	Low	0.01	High	98	100	2.96	2.97	2.97	3.10	7 940.4
79	High	Low	0.03	Low	66	93	1.89	1.90	1.90	1.89	19 917.4
80	High	Low	0.03	High	80	95	1.89	1.85	1.81	1.91	5 640.5
Domed (true) versus asymptotic fishery selectivity (alternative)											
81	Low	None	0.01	Low	100	100	1.51	1.51	1.51	2.93	30 994.1
82	Low	None	0.01	High	100	100	2.62	2.62	2.62	12.30	7 455.3
83	Low	None	0.03	Low	100	100	0.77	0.77	0.77	1.55	21 234.0
84	Low	None	0.03	High	100	100	2.03	2.03	2.03	1.92	5 312.0
85	Low	Low	0.01	Low	100	100	1.32	1.32	1.32	2.65	32 003.9
86	Low	Low	0.01	High	100	100	2.69	2.69	2.69	10.78	7 787.4
87	Low	Low	0.03	Low	100	100	0.81	0.81	0.81	1.19	21 186.6
88	Low	Low	0.03	High	100	100	1.34	1.34	1.34	2.01	5 274.8
89	High	None	0.01	Low	100	100	2.42	2.42	2.42	2.80	30 994.1
90	High	None	0.01	High	100	100	6.90	6.90	6.90	9.91	7 455.3
91	High	None	0.03	Low	100	100	1.47	1.47	1.47	1.80	21 234.0
92	High	None	0.03	High	100	100	2.70	2.70	2.70	2.31	5 312.0
93	High	Low	0.01	Low	100	100	2.72	2.72	2.72	3.04	32 003.9
94	High	Low	0.01	High	100	100	5.14	5.14	5.14	10.93	7 787.4
95	High	Low	0.03	Low	100	100	1.48	1.48	1.48	1.69	21 186.6
96	High	Low	0.03	High	100	100	2.41	2.41	2.41	2.03	5 274.8

variability and their interaction were highly significant ($P < 0.01$). In the ANOVA of the logarithm of the MSRE ratios, no factors were significant ($P < 0.05$).

In the second experiment (Table 2), both model selection criteria were almost perfect in selecting the correct model for the scenario with age-dependent natural mortality coupled with asymptotic selectivity. Both criteria made mistakes with stocks 44 and 46 for the same particular replicates. In the ANOVA of the logit values, the factors data variability and natural mortality and their interaction were marginally significant ($P < 0.1$) and the interaction between recruitment variability and fishing mortality trend was also marginally significant ($P < 0.1$). There were no significant differences among the three criteria ($P < 0.1$). Both selection criteria were essentially equivalent in the accuracy of their estimates of ending biomass. In the ANOVA of the logarithm of the MSRE ratios, no factors were significant ($P < 0.1$). For the scenario with constant natural mortality coupled with domed fishery selectivity, both selection criteria were perfect in selecting the correct model.

In the third experiment (Table 3), AIC made a few mistakes with most stocks when selecting the correct model for the scenario with asymptotic fishery selectivity. BIC was almost perfect. In the ANOVA of the logit values from the AIC and BIC selections, the factors data variability and selection criterion and the interaction between data variability and recruitment variability were highly significant ($P < 0.01$). The factors recruitment variability and fishing mortality trend and the interactions of data variability with fishing mortality trend and with selection criterion were also significant ($P < 0.05$). The AIC and BIC selections were essentially equivalent in the accuracy of their estimates of ending biomass. In the ANOVA of the logarithm of the MSRE ratios, no factors were significant ($P < 0.05$). For the scenario with domed fishery selectivity, both selection criteria were perfect in selecting the correct model.

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Discussion

Results from the experiments were generally as anticipated. Both AIC and BIC performed very well at selecting the correct model form. Across all three experiments, AIC chose the correct model for 98.8% of the replicates and BIC chose the correct model for 98.2% of the replicates. In general, the use of either AIC or BIC resulted in modest improvements in the accuracy of the estimates of ending biomass. For example, the relative error in the estimates of ending biomass ($\sqrt{MSRE}$) was 15.3% on average across all three experiments when the correct model form was selected, whereas it was 21.1% on average when the incorrect model form was selected. Thus, on average, accuracy improved by about 27% when the correct model form was selected in our particular experiments.

Although the correct model usually produced more accurate estimates of ending biomass, the degree of improvement varied by experiment. Across all three experiments, the MSRE values from the incorrect models on average were 1.83 times the MSRE values from the correct models. However, for stock 22 (experiment 1, nonequilibrium scenario), the MSRE value for the incorrect model was 19.2 times the MSRE value from the correct model. For most other stocks in this set (stocks 17–32), the incorrect model also produced very inaccurate estimates of ending biomass, even though the ending biomass was composed primarily of survivors from the 16 new sets of recruits that joined the stock during the simulated period. In general, an incorrect assumption of an equilibrium initial age composition produced much less accurate estimates of ending biomass than did an incorrect assumption of a nonequilibrium initial age composition. Many assessments of U.S. west coast groundfish have used Stock Synthesis program models with equilibrium initial age compositions. Our results suggest that such an approach could produce very biased estimates of ending biomass and that models with nonequilibrium initial age compositions should also be considered.

Using domed selectivity in experiment 3 when selectivity was asymptotic produced small losses in accuracy, whereas using asymptotic selectivity when selectivity was domed produced substantial losses in accuracy. This and the results from experiment 1 suggest that using overly complex models is likely to produce larger gains in accuracy than using overly simple models, even though the more complex models have fewer degrees of freedom.

The incorrect models in the experiments sometimes produced estimates of ending biomass that were more accurate than those from the correct model. For 19% of the experimental treatments, the MSRE values from the incorrect model were smaller than the MSRE values from the correct model. This is similar to the phenomenon that biased estimators can sometimes produce more accurate estimates than unbiased estimators.

BIC selected simpler models more frequently than AIC, which conforms to the general tendency of BIC to be a conservative model selection criterion (Kass and Raftery 1995). With regard to producing accurate estimates of ending biomass, AIC was slightly superior to BIC. Across all three experiments, the ratios of the MSRE values from the selected models over the MSRE values from the correct models ranged from 0.967 to 1.044 for AIC (mean = 1.002) and from 0.977 to 1.280 for BIC (mean = 1.004).

A limitation of our experiments is that they considered a very restricted set of stock parameters. For example, our factorial experiment did not investigate stocks that were very long-lived or very short-lived. Another limitation is that our experiments considered only two alternative models, whereas in real applications, there can be numerous alternatives. Also, our experiments ignored various subjective aspects of the model building process. For example, users of Stock Synthesis program models must determine the parameters to be fixed and the ones to be estimated. Also, the biological characteristics of the stocks often are not fully understood and configurations of the Stock Synthesis program models are based on speculation. Furthermore, Stock Synthesis program model users must choose emphasis factors that scale the various likelihood components. The emphasis factors are confounded with the age composition sample sizes and the standard deviations for the observation errors in the survey biomass estimates and the fishing effort. In our experiments, we specified the emphasis factors so that they conformed to likelihood theory (eqs. A10–A12). In real applications, users do not know correct values for the emphasis factors and have little theoretical basis for choosing them.

One goal of using statistical model selection criteria is to remove the subjective aspects of modeling. However, all models require prior knowledge based on some reasonable foundation. Objectivity, therefore, should not dictate model building but can only serve as a goal.

Acknowledgments

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Appendix A. Overview of the Stock Synthesis program and approach

The Stock Synthesis program models the dynamics of an age-structured stock using deterministic equations that can be configured to mimic a wide variety of situations. In our application with the program, we assume that fish survivorship conforms to the following exponential decay function:

$$\text{(A1)}\quad N(y+1,a+1) = N(y,a)\exp[-M-F(y)S(a)]$$

where $N(y,a)$ is the number of age a fish at the start of year y, M is instantaneous rate of natural mortality, $F(y)$ is the instantaneous rate of fishing mortality in year y for fully selected ages, and $S(a)$ is the selectivity for fish of age a. Fully selected ages are those for which $S(a)$ is unity. The Stock Synthesis program allows several flexible representations of selectivity. We used the double-logistic function

$$\text{(A2)}\quad S(a) = s_1(a)s_2(a)/max$$

$$\text{(A3)}\quad s_1(a) = \frac{1}{1+\exp[-b_1(a-t_1)]},\ s_2(a) = \frac{1}{1+\exp[b_2(a-t_2)]}$$

where b_1 and t_1 are respectively the slope and the inflection age for the ascending portion of the curve, b_2 and t_2 are respectively the slope and the inflection age for the descending portion of the curve, and "max" is the maximum of the product of s_1 and s_2 over the range of ages in the modeled stock. A dome-shaped selectivity function is attained when the parameter b_2 is positive. When b_2 is zero, the selectivity function is described as being asymptotic. The number of fish in the oldest (terminal, T) age-class is given by

$$\text{(A4)}\quad N(y,T) = N(y-1,\ T)\exp\ \{-[M + S(T)\ F(y-1)] + N(y-1,\ T-1)\exp\{-[M + S(T-1)\ F(y-1)]\}.$$

The catch in numbers of age a fish in year y is given by

$$\text{(A5)}\quad C(y,a) = \frac{N(y,a)\ S(a)\ F(y)}{M + S(a)\ F(y)}[1 - \exp[-M - S(a)F(y)].$$

The total catch biomass (the yield) in year y is given by

$$\text{(A6)}\quad Y(y) = \sum_a C(y,a)W(a)$$

where $W(a)$ denotes the average weight of age a fish in the fishery.

The Stock Synthesis program, as with all catch at age methods, requires auxiliary information for tuning the analysis in the form of independent survey indices of stock biomass or numerical abundance or data series for fishing effort or catch per unit effort (Megrey 1989 and the literature cited therein). If survey biomass data are used and the survey is conducted at the beginning of the year, as in our simulations, then the expected value of the survey biomass index is given by

$$\text{(A7)}\quad E[B'(y)] = Q'\sum_a N(y,a)W'(a)S'(a)$$

where Q' denotes the survey catchability coefficient, $W'(a)$ is the average fish weight at age a in the survey, and $S'(a)$ is the survey selectivity coefficient for age a fish. If fishing effort data (f) are used, the expected value of the effort is related to the rate of fishing mortality by

$$\text{(A8)}\quad F(y) = Q \cdot E[f(y)]$$

where Q denotes the fishery catchability coefficient.

The fitting process of the Stock Synthesis program is based on maximizing the value of the log-likelihood function for a set of parameters that define the population structure and dynamics, given all the available data. Because there are many different data types, the total log-likelihood (L_{tot}) consists of several components (L_j), each of which is attributed to a particular data type:

$$\text{(A9)}\quad L_{tot} = \sum_j L_j e_j.$$

Because different data types can be subject to different levels of observation error, the user must assign emphasis factors [e_j] that give weight to the individual components.

If age determination is exact and simple random samples of fish are taken from the fishery or the survey, then the age composition data are distributed as multinomial random variables and the log-likelihood component for these data is given by

$$\text{(A10)}\quad \sum_y J(y)\sum_a \{P(y,a)\ \log(E[P(y,a)] - P(y,a)\ \log[P(y,a)]\}$$

where $J(y)$ is the sample size for year y, $P(y,a)$ is the observed proportion at age a in the sample for year y, and $E[P(y,a)]$ is the true proportion at age a in the sample for year y.

If the survey biomass and fishing effort estimates are lognormally distributed, as in our simulations, then the log-likelihood components for these data are given by

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$$(A11)\quad \sum_{y} -\frac{1}{2}\{\log(B'(y)/E[B'(y)])/\sigma S(y)\}^2 - \log[\sigma S(y)]$$

$$(A12)\quad \sum_{y} -\frac{1}{2}\{\log(f'(y)/E[f'(y)])/\sigma F\}^2 - \log(\sigma F)$$

where $B'(y)$ and $f'(y)$ are the observed survey biomass and fishing effort estimates in year y, $E[B'(y)]$ and $E[f'(y)]$ are the corresponding expected values, and $\sigma S(y)$ and σF are the true log-scale standard deviations for these data.

To begin its iterative search for parameter values that maximize the total log-likelihood, the Stock Synthesis program needs initial estimates for the given set of parameters, which can come from prior estimates or from trial and error.

Appendix A reference

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Appendix B. Stock Synthesis program configuration and simulated data for the study

To keep our experiments simple, we simulated data for a single fishery and a single survey. The fishery data consisted of 16-year series of observations of annual total catch, age compositions, and nominal fishing effort. The survey data consisted of annual stock biomass estimates and age compositions. The fishery and the survey data were generated to be variable but unbiased with the following properties: the catch biomass data were lognormally distributed with a 10% relative accuracy (arithmetic scale), the age composition data were multinomially distributed and were not subjected to age-reading errors, the fishing effort data were lognormally distributed with mean value equal to F/Q and a fixed coefficient of variation for all years, the survey biomass data were lognormally distributed with mean $Q'\Sigma_a N_{y,a} W'_a S'_a$ and a fixed coefficient of variation for all years, and the weight at age data were generated in a deterministic manner using

$$(B1)\quad W(a) = 0.0001\{10[1 - \exp(-0.2a)]\}^3.$$

The lognormal random data were generated in a manner that accounted for logarithmic transformation bias.

The Stock Synthesis program was configured to assume that individual data sets had the following properties: the age composition data were multinomial random variables and had no age-reading errors, the fishing effort data were lognormally distributed and the program was given the true log-scale standard deviation for these data (σF), the survey biomass estimates were lognormally distributed and the program was given the true log-scale standard deviation for these data (σS), the survey selectivity function was asymptotic, and weight at age data were exact. The likelihood components for the different data sources were all given emphasis factors of 1 for computing the total likelihood.

In all of the generated data sets, the simulated fish stocks were unfished prior to the start of the simulation and then were subjected to an instantaneous rate of fishing mortality of 0.07·year^{-1} during the first year, with F increasing a fixed amount at the start of each year thereafter. The true fishery catchability coefficient and the true survey catchability coefficient were constant throughout each simulated period ($E[Q] = 0.003$, $E[Q'] = 0.1$). There was no sexual dimorphism in the simulated stock.

Errorless data were generated and analyzed by the Stock Synthesis program to verify that the demographic equations used by the simulator were the same as those of the Stock Synthesis program. The true parameter values were provided as the initial values with which the Stock Synthesis program began its iterative search for the set of maximum likelihood parameter estimates.

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Predation on hatchery-reared lobsters released in the wild

Gro I. van der Meeren

Abstract: Predation on hatchery-reared lobsters (*Homarus gammarus*) in the wild was studied in order to identify predators in southwestern Norway on rocky and sandy substrates in winter and summer. Lobsters of 12–15 mm carapace length were tagged with magnetic microtags. About 51 000 juvenile lobsters were released on 10 occasions at three locations. Predator samplings were by trammel nets, eel traps, and videorecordings during the 24 h immediately following the releases. In summer, loss to predators occurred on both rocky and sandy substrates. The loss was lower in winter when lobsters were found as prey in predators caught on sand. The risk of fish predation was highest in the first hours after release, when the lobsters were out of shelter. The wrasses *Labrus bergylta* and *Labrus mixtus* were the major predators of lobsters, while Atlantic cod (*Gadus morhua*), shorthorn sculpin (*Myoxocephalus scorpius*), and crab (*Cancer pagurus*) were mainly winter predators. Winter predators were never as abundant as summer predators. To minimise predatory loss of reared and costly lobsters, they should be released onto rocky substratum in winter. Due to the damage to the predated lobsters, it was not possible to correlate survival against lobster size.

Résumé : Nos études, menées dans le sud-ouest de la Norvège sur la prédation exercée dans le milieu naturel sur des homards d'élevage (*Homarus gammarus*), visaient à identifier les prédateurs sur des substrats rocheux et sableux, en hiver et en été. Des homards dont la longueur de carapace était de 12–15 mm ont été étiquetés avec des micromarques magnétiques. Environ 51 000 homards juvéniles ont été lâchés en dix occasions à trois endroits. Pour échantillonner les prédateurs, on a eu recours à des trémails, des pièges à anguille et des enregistrements vidéo pendant les 24 h qui suivaient immédiatement les lâchers. En été, la prédation se produisait à la fois sur les substrats rocheux et sur les substrats sableux. Elle était moins intense en hiver, les prédateurs ayant consommé des homards se retrouvant surtout sur le sable. Le risque de prédation par des poissons était au maximum dans les premières heures qui suivaient le lâcher, lorsque les homards étaient dépourvus d'abris. Les labres *Labrus bergylta* et *Labrus mixtus* étaient les principaux prédateurs des homards, tandis que la morue (*Gadus morhua*), le chaboisseau (*Myoxocephalus scorpius*) et le crabe (*Cancer pagurus*) étaient surtout des prédateurs d'hiver. Les prédateurs d'hiver n'ont jamais été aussi abondants que les prédateurs d'été. Pour minimiser les pertes par prédation des coûteux homards d'élevage, il faudrait les lâcher en hiver sur des substrats rocheux. Étant donné les dommages subis par les homards soumis à la prédation, il n'a pas été possible de corréler la survie à la taille des homards.

[Traduit par la Rédaction]

Introduction

Releases of reared juvenile lobsters (*Homarus* sp.) have been made for more than 100 years on both sides of the North Atlantic Ocean (Addison and Bannister 1994), including Norway (Appelöf 1909; Dannevig 1928; Tveite and Grimsen 1995). Through the development of the magnetic binary-coded microtag, it is possible to tag lobster juveniles before release into the sea for recognition years later in commercial landings (Jefferts et al. 1963; Wickins et al. 1986). A series of releases of tagged juvenile lobsters have since been conducted in Europe (Latrouite and Lorec 1991; Bannister et al. 1994; Agnalt et al. 1999).

In Norway, a large-scale lobster hatchery at Kyrksæterøra was built in 1973 to produce juvenile European lobsters (*Homarus gammarus*) for stock enhancement and commercial sea ranching (Grimsen et al. 1987). Since 1988, the Institute of Marine Research has studied the potential benefit of releasing juveniles into local lobster stocks (Borthen et al. 1998).

Information on predators of juvenile European lobsters is scarce and is usually based on anecdotes and not quantified observations during release of reared juveniles (Howard 1983; van der Meeren 1991). The aim of this paper is to identify seasonal variation in lobster loss to predators in the first 24 h following release, compare lobster loss to predators after release to rocky versus sandy bottom, and identify species of lobster predators and their occurrence and activity as predators during different seasons. Recommendations to reduce loss of juvenile lobsters in future releases will be made.

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Materials and methods

Study sites

The research program, conducted between 1988 and 1994, consisted of two main components. First, there were a series of large-scale releases to evaluate the possibility of enhancing local lobster stocks, and hence the fishery (Agnalt et al. 1999). This project component was undertaken within the context of the Norwegian Sea Ranching Programme (PUSH) and in cooperation with local

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fishermen. Lobsters were released at the complex archipelago of Kvitsøy, which has approximately 81 700 km of shoreline (Fig. 1). The project's second component was designed to evaluate how, when, and where to release lobster juveniles so as to minimize postrelease loss. These latter releases took place at selected locations at Øygarden and Huftarøy, Hordaland County, Norway (Fig. 1). The releases at Øygarden were conducted in Langøysundet, a narrow sound with shorelines exhibiting well-defined areas of rocky substrate covered with boulders and cobble (rocks) and also areas with soft bottoms (sand). Lobsters were released onto each habitat type. At Huftarøy, lobsters were released by divers onto three rocky areas simultaneously and in patches of high density.

Techniques for low-cost transportation and large-scale (>20 000 individuals·day^{-1}) release of lobster juveniles, as well as lobster behaviour in the first minutes after a release, have been described elsewhere (van der Meeren 1991, 1993).

Tagging and release techniques

Lobsters were hatched and reared at the large-scale lobster hatchery, Kyrksæterøra, Norway. Lobsters released from 1990 to 1994 were tagged with magnetic binary-coded microtags (North West Marine Technology Inc.) at 5–8 months of age, which represented a carapace length of approximately 12–15 mm. Older lobsters (12–24 months old, 22–32 mm carapace length), released in August 1988, were marked by burning small spots in their carapace (Abrahamsson 1965). They were packed in damp transportation boxes and cooled with ice and and arrived at the release location within 18 h. On arrival, lobsters were given a minimum recovery time of 30 min in ambient seawater. They were then released into the sea surface in shallow waters (<10 m). All individuals were released within 3 h of opening the transportation boxes. Transport-related mortality was <5%.

Releases were conducted in summer (May, June, and August) and winter (December, February, March, and April). The release densities at both Langøysundet and Kvitsøy were not more than one lobster per 1 m of running shoreline, as recommended by the Institute of Marine Research research team. However, the fishermen charged with conducting some of the releases often did so at higher densities, especially in areas that they thought were "good lobster places."

Approximately 51 200 juvenile lobsters were released during this study. A total of 10 releases were done: four at Langøysundet, four at Kvitsøy, and two at Huftarøy (Table 1). The releases in Langøysundet consisted of three winter releases totalling 3850 lobsters and one summer release of 2100 lobsters. Winter releases of 35 688 lobsters, from 11 000 to 29 000 juveniles each year, were conducted at Kvitsøy, and two summer releases totalling 9500 lobsters were conducted at Huftarøy.

Winter releases at Kvitsøy were conducted by local lobster fishermen within their own established fishing grounds. Rocky substrates were favoured, but due to the complex and variable bottom topography and the high numbers of lobsters for release, individuals were probably also released onto sand. On one occasion (April 1993), the releasers were instructed to place groups of lobsters on rocky substrate and on sand for comparison (Table 1). Summer releases were not conducted at Kvitsøy. Divers were used occasionally to determine whether predation was a factor during winter releases in March at Kvitsøy and Langøysundet.

For the August 1988 summer release at Huftarøy, lobsters were released at 3–6 m depth onto rocky substrate. They were released directly from the transportation boxes without acclimatisation. Divers observed the lobsters on the bottom for 45–60 min following this release.

The June 1991 release at Huftarøy was conducted to make videorecordings. These lobsters were acclimated to ambient seawater temperature in open trays for at least 1 h and were released one or two at a time over a seaweed-covered sand bottom. A diver with a videocamera was situated on the bottom and recorded both the descent of the lobsters and the activity on the sea bottom. One series of releases was conducted at 1 m depth along a portion of the shore where the sandy and rocky bottom met, while the rest of the releases were at depths of 3 m on sand covered with kelp.

Predator sampling

The predation records and counts of eaten lobsters/lobster tags were made using 59 trammel net hauls, six eel trap hauls, approximately 20 min of videorecordings, and direct observation done by divers (Table 2). Similar trammel nets were available at all locations and were used to provide comparable data on the diversity of swimming organisms and potential predators. Eel traps were used on an occasional basis to fill in catches of bottom-dwelling species too small to be caught in trammel nets but still potential lobster predators. The sampling at Langøysundet was undertaken using trammel nets set during the 24 h after each release. The nets covered more than half of the release area, and the number of nets varied according to the size of the release. The trammel nets were 2 × 28 m with a fine mesh of 70 mm and a coarse mesh of 460 mm.

At Kvitsøy, sampling was done during the 24 h after each lobster release. Due to the complex bottom structures at Kvitsøy, a mixture of the two bottom types could be found nearly everywhere, but the sampling was done by systematic fishing in eight selected areas: four dominated by rocks and four with more sand. Eight trammel nets, similar to those deployed at Langøysundet, were used in the same locations immediately after each release once a month from April 1993 to April 1994. In addition, the one instance from the monthly fishery where a lobster predator was present is included in this paper.

At Huftarøy, predator sampling was on rocks only, with one trammel net and two eel traps deployed at each of the three locations immediately after the release in August 1988 and then once a week for 4 more weeks. The three-chambered eel traps were circular, 1.5 × 0.45 m, with a 3.5 × 0.45 m leading net at the entrance. Mesh size of the leading net and first chamber was 35 mm, 30 mm in the second chamber, and 25 mm in the third chamber. Sampling was undertaken directly on the release sites and not in the surrounding areas.

Surface sea temperature was measured at release or was obtained from routine monitoring established at certain locations. These data were used to evaluate whether sea temperature and lobster predation were correlated.

Statistics

A χ^2 test was used to compare the distribution of numbers of animals from the most important predatory groups in the samples (catch composition) during winter and summer at each location (H_0: no difference in the species composition between summer and winter at any given location). The same test was used to make between-location comparisons (H_0: no difference in the species composition between locations in either winter or summer).

Possible between-season (and bottom substrate) differences in predation were evaluated using the Fisher exact probability test and the χ^2 test. (H_0: no differences in predation on lobsters on rocky and sandy bottom in winter and summer). Each of the release locations was tested separately due to small differences in release technique between teams as well as regional differences in bottom substrates and local fauna.

Spearman rank correlations (r_s) were used to test the relationship between sea temperature and catch (fish and crustaceans) per net per day (CPUE), eaten lobster juveniles or lobster tags per net per day (LPUE), and numbers of individual predators per net per day (PPUE) using data from all three locations (H_0: no differences in catch of fish, number of lobsters eaten, or number of lobsters per predator at different temperatures). The correlations were based on

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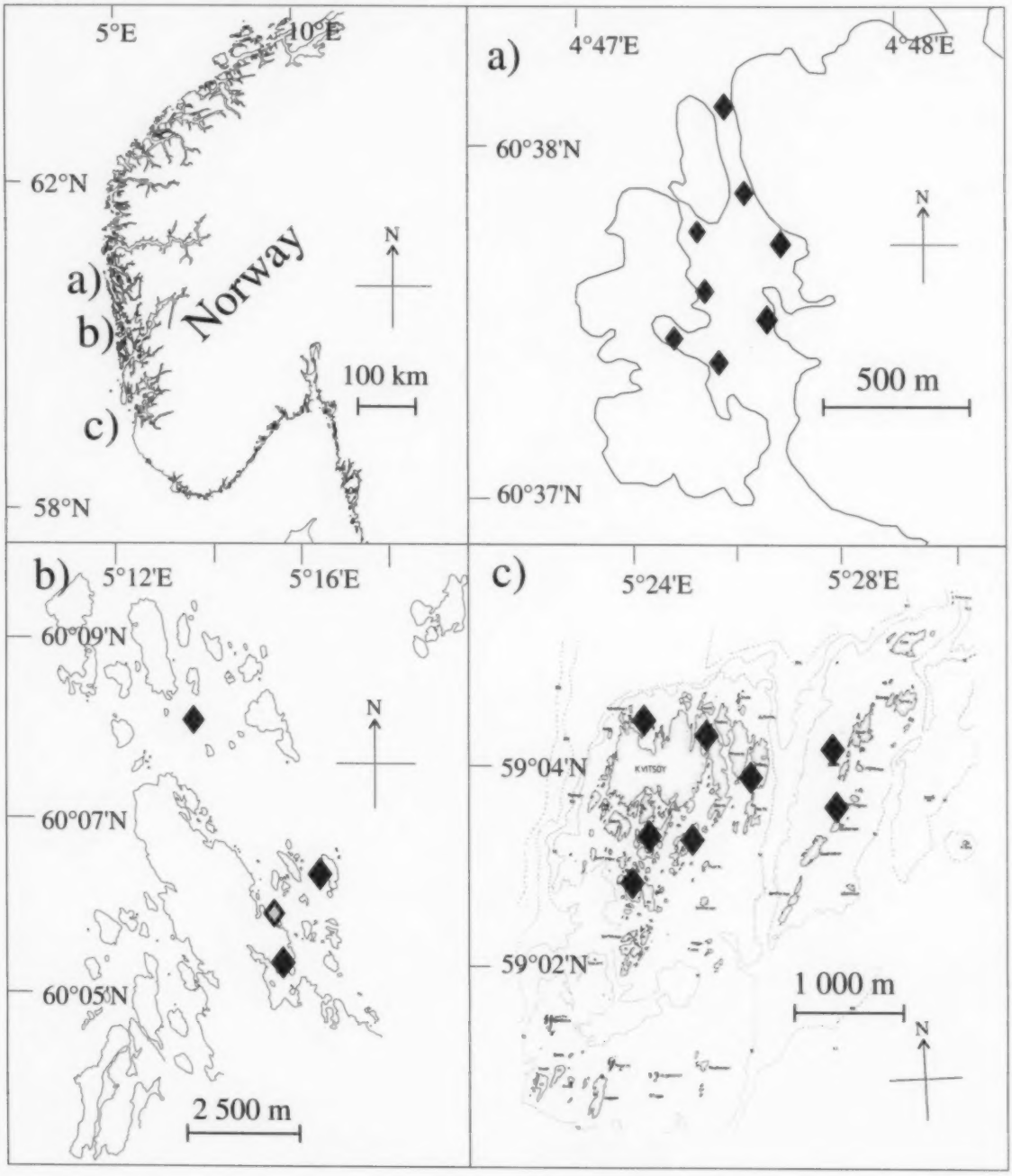
Fig. 1. Map showing (*a*) Langøysundet (4°47′E, 60°38′N), (*b*) Huftarøy (5°14′E, 60°07′N), and (*c*) Kvitsøy (5°26′E, 59°04′N) in southwestern Norway. Predator sampling areas are indicated with black diamonds. The grey diamond indicates the videorecording area. In Langøysundet, from three to eight selected areas were fished simultaneously according to the numbers of released lobsters. At Huftarøy and Kvitsøy, all areas were fished simultaneously.



the nine samplings associated with the lobster releases for stock enhancement purposes. The data from June 1991 and June 1993 were therefore omitted.

A one-tailed Student's *t* test on log-transformed size measurements was used to compare the size of Atlantic cod (*Gadus morhua*) with versus without lobster remnants in the gut. In cases for which the size distributions were not normally distributed, the one-tailed Mann–Whitney *U* test was used to evaluate size differ-

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Table 1. Numbers of lobsters released and trammel nets used in the predator sampling.

Location	Date	Lobsters released			Number of nets		
		Sum	Rocks	Sand	Sum	Rocks	Sand
Langøysundet	3 Dec. 1992	750	0	750	4	0	4
	25 Feb. 1993	2 100	1600	500	6	4	2
	27 Mar. 1992	1 000	1000	0	6	6	0
	21 May 1992	2 100	1400	700	8	4	4
	Sum	5 950	4000	1950	24	14	10
Huftarøy	24 June 1991	75 – 100	0	75 – 100			16:10 min (video)
	17 Aug. 1988	9 500	9500	0	9	9	0
Kvitsøy	7 Dec. 1993	5 576			8	4	4
	10 Mar. 1994	14 160			8	4	4
	18 Apr. 1994	4 168			8	4	4
	23 Apr. 1993	11 784	5897	5897	8	4	4
	Sum	35 688			32	16	16

ences between other lobster predators and their conspecifics (H_0: individuals that preyed upon lobsters were not different in size from their conspecifics that did not prey upon lobsters).

Results

Season

The summer release at Huftarøy in August resulted in substantial losses of lobsters. When lobsters were released without prior acclimatisation, as in this case, they were either passive for at least 15 min, swimming vertically up and down in the water column, or climbing to the top of rocks and kelp. This was in gross contrast with later releases. When divers inspected parts of the release areas 30 min after release, the acclimatised lobsters had moved into shelter. Only on a few occasions could parts of antennae, claws, or tails be seen.

Divers commonly saw attacks by predators during summer releases, but not winter releases, at Kvitsøy and Langøysundet. The visual impression from Huftarøy was that Labridae were the dominant predators, and massive attacks on lobsters by the wrasse *Labrus bergylta* were recorded in June. Within 16 min, 17 attacks and nine killings were registered. In August 1988, reports from divers described numerous fish, mostly Labridae, attacking and eating lobsters within minutes after the release. At all three locations, divers estimated the immediate loss to be more than 10% of the lobsters within the first hour. Despite the limited fishing effort (Table 1), 0.17% of the released lobsters were found in the stomachs of fish the 24 h following the release (Table 2). Divers also reported crushed lobsters in Labridae feces at one of the release locations the next day, but no live lobsters. A significantly higher fraction of the released lobsters were eaten after the summer release at Langøysundet compared with winter releases at the same location (χ^2 = 13.203, df = 1, $p < 0.0001$). Relative to the numbers of lobsters released in winter at Kvitsøy and Langøysundet, less lobsters were found eaten at Kvitsøy than at Langøysundet, 0.02 and 0.16%, respectively (Tables 1 and 2).

Sea temperature, CPUE, LPUE, and PPUE were not correlated (CPUE: $r_s = 0.185$, $n = 9$; LPUE: $r_s = 0.411$, $n = 9$; PPUE: $r_s = 0.377$, $n = 9$) (Fig. 2).

Bottom substrate

Significantly higher numbers of lobsters were found eaten by fish and crustaceans caught on sand than on rocks, both at winter releases at Langøysundet (73.7%; Fisher exact probability test, $p = 0.0012$) and at Kvitsøy (87.5%; Fisher exact probability test: $p < 0.0001$). The predation in the summer at Langøysundet, as recorded from stomach analyses, and the visually observed predation at Huftarøy occurred with much less difference both on rocks and on sand (August 1988 and June 1991; Fisher exact probability test, $p = 0.814$) (Table 2).

Predators

The highest CPUE in the trammel net fishery occurred in the summer, with 32.5 at Kvitsøy and 13.3 at Hufthamar (Fig. 2). CPUE at Langøysundet fluctuated between 4.2 and 13.0, but both the highest and lowest CPUE were after winter releases. CPUE at Kvitsøy in winter were similar to those at Langøysundet and went from 5.8 to 11.9.

In winter, LPUE and PPUE were one to one, except for a December release at Langøysundet, while in summer, LPUE were higher than PPUE (Fig. 2). This difference was mainly due to three predatory species, *L. bergylta*, *G. morhua*, and European flounder (*Platichthys flesus*), often found with two or more lobsters eaten per fish. Usually, only one lobster per predator was found after winter releases, except for two lobster tags found in one shorthorn sculpin (*Myoxocephalus scorpius*) stomach in April 1993 and six lobsters found in one *G. morhua* stomach in December 1992. No correlation between the size of the released lobsters and that of the eaten lobsters could be made due to the conditions of the eaten lobsters, being crushed and more or less digested by the predator.

Of the 32 species registered in the predator sampling, 29 were caught in the summer and 24 in the winter (Table 3). Nine of these species, mainly the Labridae, showed predator

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Table 2. Captures of fish and crabs that had eaten lobsters and numbers of eaten lobsters recovered.

		Predators			Eaten lobsters		
Location	Date	Sum	Rocks	Sand	Sum	Rocks	Sand
Langøysundet	3 Dec. 1992	1	0	1	6	0	6
	25 Feb. 1993	0	0	0	0	0	0
	27 Mar. 1992	0	0	0	0	0	0
	21 May 1992	6	3	3	13	5	8
	Sum	7	3	4	19	5	14
	Frequency		42.9	57.1		26.3	73.7
Huftarøy	24 June 1991	15*	0	15*	9*	0	9*
	17 Aug. 1988	8	8	0	16	16	0
	Sum	>23	8	>15	25	16	9
	Frequency		34.8	62.5		64.0	36.0
Kvitsøy	7 Dec. 1993	1	0	1	1	0	1
	10 Mar. 1994	1	0	1	1	0	1
	18 Apr. 1994	4	1	3	5	1	4
	23 Apr. 1993	1	0	1	1	0	1
	Sum	7	1	6	8	1	7
	Frequency		14.3	85.7		12.5	87.5
All locations	Sum	>37	12	>25	52	22	30
	Frequency		32.4	67.6		42.3	57.7

Note: Videorecordings are marked with an asterisk. Numbers of predators were in this case not certain due to the limited frame of the videocamera. Sum is the total numbers, in each region and totally, of lobster predators and eaten lobsters recovered. Frequency is the fraction of lobster predators and eaten lobsters recovered from captures in rocky and sandy habitat.

activity only in the summer. Although the crab *Cancer pagurus* and *M. scorpius* were registered as predators only in the winter, they were found in the catches year-round. Yet 71.4% (n = 5) and 96.0% (n = 48) of *M. scorpius* from Langøysundet and Kvitsøy, respectively, were landed in the winter, indicating a high winter activity of this species (Table 4). Only two species, *G. morhua* and the crab *Carcinus maenas*, were found as active predators in both seasons. In March, the only predator recorded was *M. scorpius*, even though more than 17 000 lobsters had been released at two locations.

The summer sampling from Huftarøy and Langøysundet showed no significant differences in species composition (χ^2 = 0.4702, df = 2, $p > 0.05$) (Table 4). The difference in sampled species composition between summer and winter was highly significant, both at Langøysundet (χ^2 = 64.370, df = 2, $p < 0.0001$) and at Kvitsøy (χ^2 = 276.630, df = 2, $p < 0.0001$). While the Labridae dominated in numbers in the summer, the Gadoidae and crustaceans were most numerous in the winter. The very high density of Labridae at Kvitsøy, compared with Langøysundet/Huftarøy, gave a significant difference between sampled species composition at Kvitsøy and at the other locations (χ^2 = 9.014, df = 2, $p < 0.025$). The difference in species compositions between Langøysundet and Kvitsøy was even greater in winter, when the Gadoidae dominated at Langøysundet, while crustaceans and Cottidae were most numerous at Kvitsøy (χ^2 = 61.897, df = 4, $p < 0.001$).

Juvenile lobsters were usually not found eaten at times other than the day of release at Huftarøy or Kvitsøy. The two possible exceptions were at Kvitsøy, where one lobster released in April was later recognised by the tag in an adult lobster delivered by fishermen 1 month after release and one tag found in an *L. bergylta* more than 2 months after release.

The predator size was usually between 15 and 40 cm (Table 5). Most predators did not differ significantly in size from other individuals of their species, but exceptions were seen. In the wrasse *Labrus mixtus*, all predators were females and were significantly smaller than the males and females with no lobster remnants in the stomach (one-tailed Mann–Whitney U test, p = 0.022). The wrasse *Crenilabrus melops* predators had a tendency to be among the smaller of that species (one-tailed Mann–Whitney U test, p = 0.058), while the wrasse *Ctenolabrus rupestris* tended to be among the larger, although not significantly so (one-tailed Mann–Whitney U test, p = 0.058). The two predatory poor cod (*Trisopterus minutus*) caught were both larger than the rest encountered.

Discussion

Release and sampling techniques

When releasing juvenile lobsters after transportation in cool, damp boxes, acclimatisation to ambient sea temperature is vital to keep the lobsters awake and alert and walking forwards while using the claws and antennae to investigate potential shelters (van der Meeren 1991). However, even when acclimatised and released at the sea bottom instead of the sea surface, summer releases continued to result in higher lobster mortality than winter releases. The most plausible cause for the difference in mortality is found in the predator species composition in summer and winter. The

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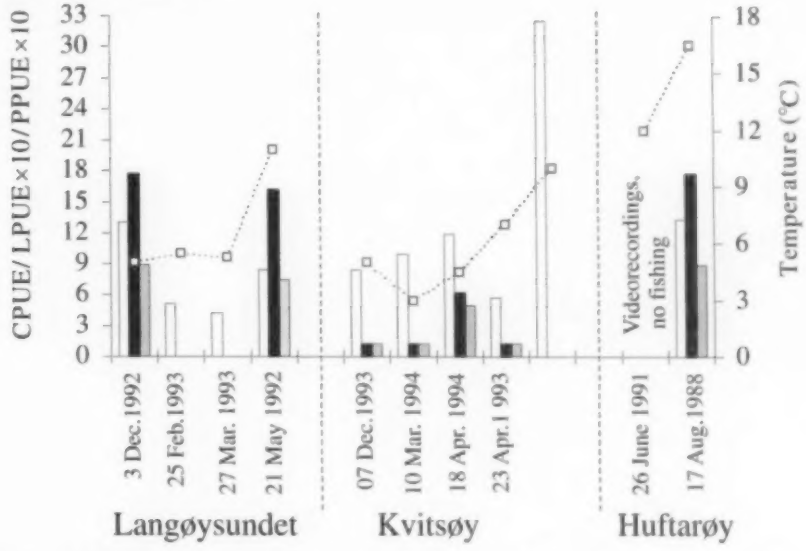
Fig. 2. CPUE (open bars), as fish/crustaceans per fishing gear, in the first 24 h after the releases of juvenile lobsters. For comparison, LPUE (solid bars) and PPUE (shaded bars) are also presented and are multiplied by 10 to fit onto the graph. Temperature (squares) at the release dates are shown, to see if predation might be temperature dependent. Trammel nets were used for predator fishing, while eel traps were used in addition at Huftarøy.



high density of Labridae in summer makes the risk of loss of newly released lobsters to predators much higher than in winter. Mortality could also be due to temperature-enhanced lobster activity, resulting in reduced shelter use and increased predator exposure (van der Meeren 1993), but this is less likely. Shelter use in juvenile American lobster (*Homarus americanus*) is sensitive to predators (Wahle 1992*b*). Underwater observations and videorecordings showed that the acclimatised lobsters were seeking shelter within a few minutes after release even in the winter. No acclimatised lobsters could be seen on the sea bottom by divers 30 min after release at any time of the year.

The magnetic steel tag in a released lobster made it easily identifiable even in the stomach of a live fish when checking the catch with a tag detector. Even when the lobster had been dissolved, the tag would reveal age and origin of the lobster. Due to the tags, the quantification of lost lobsters in the samples should be reliable, although it might be underestimated due to lobsters and tags being discarded from the predators before examination. Lobster remnants in Labridae feces were observed less than 24 h after the release in August.

Bottom substrate and season

Even though the samplings were not designed to catch all the lobster predators but to present a subsample of the present fauna, this study showed that both the time of year and the bottom substratum are of importance for survival of newly released juvenile lobsters. The distribution of predators could be confused by fish swimming between the bottom types. Yet significantly more predators were captured on sand in winter, in spite of the fact that most of the lobsters were released onto rocks, and one of the important winter predators, Cottidae, is known to be a rock-preferring species (Pethon 1985). This difference in lobster predation between rocks and sand was not found in summer, but that might be due to the change in predator species from bottom-dwelling fish to more mobile species. The Labridae, visually observed when eating lobsters in the summer, seemed to be attracted into the release area. It could also be due to the feeding activity of conspecifics, by the presence of the lobsters, or by the human activity in connection to the releases. Specific substrates, usually cobble grounds, are important nursery grounds for juvenile *H. americanus* (Wahle and Steneck 1991; Wahle 1992*a*, 1992*b*). The vulnerability of juvenile lobsters out of shelter has been demonstrated in *H. americanus* (Lavalli and Barshaw 1986; Barshaw and Lavalli 1988; Wahle and Steneck 1992) and also recently in *H. gammarus* (Mercer et al. 2000).

Except for two cases, all the eaten lobsters in this study were found within 24 h after release. This indicates that predators are a hazard mainly to lobsters immediately after release, before they are established within shelters. The major predator groups of *H. americanus* are similar to the predators found in tl.is study, with Labridae, Cottidae, and crustaceans being the most important predatory taxa. In addition, this study found that the Gadoidae were among the important predators. More species than those presented here have been observed attacking tethered early benthic lobsters in European waters (Mercer et al. 2000).

A remaining unsolved problem is to find the optimal shelter habitats for juvenile *H. gammarus*. Distribution of settling juveniles in several Brachyura species is caused by habitat selection (Eggleston and Armstrong 1995; Hedvall et al. 1998). Wild juveniles have never been found in Europe either in cobble or in any other habitat (Mercer et al. 2000). On the other hand, the fauna living in cobble grounds in Europe is much more diverse than that in North America (Wahle 1998; Mercer et al. 2000). To develop successful

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Table 3. Fauna composition during times of lobster predation.

Species	Dec.[a,b]	Feb.[a]	Mar.[b]	Apr.[b]	May[a,c]	June[b,d]	Aug.[d]
Cancer pagurus	■			■			
Gadus morhua	■	▒	▒	▒	■	▒	▒
Myoxocephalus scorpius	▒	▒	■	■	▒	▒	▒
Trisopterus minutus	▒						■
Carcinus maenas	▒		▒	■		■	■
Ctenolabrus rupestris	▒			▒	▒	▒	■
Labrus mixtus	▒				■	▒	■
Crenilabrus melops	▒				▒	▒	■
Platichthys flesus			▒		■	■	■
Labrus bergylta				▒	■	■	■
Homarus gammarus				▒	■	▒	▒
Anguilla anguilla							■
Gobius niger							
Pollachius pollachius	▒	▒	▒	▒	▒	▒	▒
Pollachius virens	▒	▒		▒	▒	▒	▒
Galathae spp.	▒		▒	▒			
Agonus cataphractus	▒				▒	▒	
Lithodes maja	▒						
Pleuronectes platessa		▒		▒			
Cyclopterus lumpus			▒	▒		▒	
Microstomus kitt			▒	▒			
Lycodes vahlii			▒	▒		▒	
Hyas araneus			▒	▒			
Clupea harengus			▒				▒
Pagurus spp.				▒			
Anarhicas lupus				▒			
Callionymus lyra					▒		
Trachinus draco					▒		
Merlangius merlangus					▒		▒
Pomatoschistus minutus							▒
Raniceps raninus							▒
Centrolabrus exoletus							▒

Note: Grey box, species registered; black box, species registered as lobster predators (lobsters/lobster tags in the stomach or mouth); open box, species not registered. Sampling methods were

[a]Trammel nets at Langøysundet.
[b]Trammel nets at Kvitsøy.
[c]Lobster pots at Kvitsøy.
[d]Diver observations, eel traps, and trammel nets at Huftarøy.

lobster release programmes anywhere, it is important to describe the best habitats for juvenile stages in order to offer released juveniles the best possibilities for survival.

Predators

Potential predators were probably missed due to low fishing intensity in relation to the area into which the lobsters were released, gear selectivity, choice of fishing location, or low density of that species compared with fishing effort. Some species might also be potential predators on lobsters of a size another than that of the released lobsters. The lobsters released were all relatively large juveniles in the early benthic phase and some near transition to the adolescent phase in August 1988. *Ctenolabrus rupestris* and *T. minutus* found as predators after this release tended to be among the larger of these species. It is plausible that smaller sized fish of both species would succeed in eating smaller lobsters than the released ones. The data set assembled in this study was not fit for statistical tests of the relationship between predator and lobster size. The lobsters were produced to meet the standard set by the Institute for Marine Research for stock enhancement purposes and not for qualitative ecological experiments. In addition, even if lobsters of various sizes were released, the remnants recovered were usually only the tags or too fragmented to be remeasured. Despite methodical flaws in order to get a more detailed picture of potential lobster predators, our documented predator list presents a series of important predators in European waters, where some are seasonally present in sufficient numbers to negatively impact the survival of released lobsters.

The Labridae, known as an important group of shallow-water predators in tropical and temperate waters (Wahle and Steneck 1992), include the most numerous fish species in Nordic European shallow waters in the summer (Hildén 1984). Representatives of this fish family were found to be the most important lobster predators in this study. These species are active only in summer and autumn in temperate waters, leaving the shallow waters in the winter (Hildén 1984).

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Table 4. Comparisons of occurrence between the four major predatory families and a fifth group, mainly composed of flatfish and eels, as total number of animals caught at each release location in the summer and winter.

	Labridae	Gadoidae	Cottidae	Decapoda	Other	Sum
Langøysundet (winter)	7	63	5	28	5	108
Predators (%)	0	1.6	0	0	0	0.9
Kvitsøy (winter)	12	36	48	102	10	208
Predators (%)	0	0	8.3	2.9	0	3.4
Langøysundet (summer)	41	18	2	0	6	67
Predators (%)	9.8	5.6	0	0	16.7	9.0
Huftarøy (summer)	86	37	1	4	7	135
Predators (%)	11.6	5.4	100	25	28.6	11.9
Kvitsøy (summer)[a]	192	57	2	7	2	260
Predators (%)	0.5	0	0	0	0	0.4

Note: The frequency of active lobster predators within each group is shown for each region.
[a]The summer sampling at Kvitsøy was not related to a lobster release.

Table 5. List of predatory fish species, with mean size, SD, and numbers of documented predators and as well as the rest of catch of the same species.

	Predators			Others		
Species	Size (mm)	SD	*n*	Size (mm)	SD	*n*
Labrus bergylta	280.0	60.0	4	271.6	61.3	81
Labrus mixtus	208.0	14.4	6	245.3	33.7	103
Crenilabrus melops	172.5	2.5	2	187.3	12.9	110
Ctenolabrus rupestris	180.0	0	1	132.1	18.8	24
Gadus morhua	280.0	50.0	2	347.1	77.3	95
Trisopterus minutus	300.0	0	2	200.0	0	2
Myoxocephalus scorpius	193.3	31.1	4	193.3	26.5	71
Anguilla anguilla	550.0	0	1	560.0	50.0	2
Gobius niger	150.0	0	1	150.0	0	1
Platichthys flesus	360.0	0	1	345.0	35.0	2

The Labridae are more or less dependent on rocky substratum (Hildén 1984), like the habitat into which many of the lobster were released. Sand occurs in patches within and below rocky shores, well within the swimming range of the Labridae, in most places along the Norwegian coast. Four out of five Labridae species were lobster predators, and *L. bergylta* and *L. mixtus* had usually eaten more than one lobster per fish. In this study, most of the Labridae were between 10 and 50 cm. The larger sized species *L. bergylta* and *L. mixtus* are both known to mainly consume decapod crustaceans. Why only female *L. mixtus* were found to be predatory might be due to the territorial males being busy fending off nonterritorial intruders, attracted into their territory by the lobster release, or to size-selective feeding by the smaller females. *Labrus mixtus* is hermaphroditic, and large dominant females in the harem turn into males after the territorial males disappear (Hildén 1984). The smaller Labridae species mainly consume amphipods and copepods (Hildén 1984) but can be expected to be predators on smaller juvenile lobsters than those released in this study.

The second important predator, *G. morhua*, is common along the Norwegian coast throughout the year (Pethon 1985). Most of the specimens caught during this study did not contain any lobsters or lobster remnants. However, the ones that did prey on lobsters had eaten several lobsters per fish. If lobsters released in high densities can induce search selectivity in these highly mobile predators, the potential for substantial loss of lobsters is high.

Crustaceans, like the Brachyura *C. pagurus* and *C. maenas*, are commonly found along the Norwegian coast (Christiansen 1969). They are known to have temperature-dependent activity, being active in the summer (Naylor 1963; Crothers 1968; Karlsson and Christiansen 1996). Yet, predation on juvenile lobsters was seen in the spring, summer, and winter. Still, the number of lobsters taken by crustacean predators was low. The videorecorded crab attacks showed that lobsters had to walk in close to the crab before the crab attacked. Crab predation was recorded on sand, but crabs can catch juvenile lobsters on rocky substrate also (Lavalli and Barshaw 1986). Predation by crabs might be cumulative over time; since they can also forage within rocky shelters, they can probably catch shelter-living lobsters in the weeks and months after the release. In addition, intraspecific competition and predation in newly settled and juvenile Brachyura species, which in many ways are comparable with lobsters, are the most common regulation of population size besides refuge habitats (Kurihara and Okamoto 1987; Pile et al. 1996; Moksnes 1999). This also seems to be true for the European lobster (Mercer et al. 2000). Long-term mortality due to intra- and inter-specific predation needs further study.

Myoxocephalus scorpius was the only species to be active

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as a predator mainly in the winter. One individual of this species was the only predator registered after the release of 17 000 juvenile lobsters in February–March. Due to the low density and its sit-and-wait foraging tactic, it will probably not contribute to lobster mortality in winter at the same scale as the Labridae do in the summer.

The small-sized members of the fish families Gobiidae, Blennidae, and Pholidae as well as *C. maenas*, *Liocarcinus arcuatus*, and other portunid and xanthid crabs are representative of known and potential predators living in the shallow waters of Europe, presenting a threat to early benthic phase lobsters, at least when the lobsters are out of shelter (Mercer et al. 2000). Rearing the lobsters to more than 15 mm carapace length might protect them from smaller fishes and crustaceans, but they are still vulnerable to predatory fishes longer than 15–18 cm. Due to the high density of the Labridae from May to October, releases of lobsters during this period can be expected to induce high mortality in the lobsters. Predator-deterrent actions could be carried out in order to diminish the numbers of predators from a location prior to releasing the lobsters. Large-scale fishing operations to remove the numerous Brachyura and Labridae living along the coast may disrupt local ecosystems and create unwanted ecological side effects. In addition, if the predators are of commercial importance themselves, such as *G. morhua* and the Labridae in some regions of Norway, removal of the fish will create conflicts with local fisheries. Since the lobsters seem to be quite safe from fish predators as soon as they find shelter, it is not necessary to remove the fish permanently. One tempting, but not tested, solution might be to scare the predators temporally away by the use of acoustic soundings a short time before the release at the chosen location (E. Ona, Institute of Marine Research, P.O. Box 1870, N-5817 Bergen, Norway, personal communication).

The numbers of lobsters found in fish and invertebrate predators in this study were quite low. When divers estimated the loss after an August release to be more than 10%, the recollection of juveniles from fish stomachs was only 0.17%. The present quantity of predated lobsters must therefore be regarded as subsamples and should not be expected to represent the total loss within 24 h from release. The actual loss could be up to 100 times the observed 0.17% mortality based on stomach contents. In addition longer term mortality must be expected, even if it was not identified in this study. Because little is known of the natural abundance of European lobsters (Mercer et al. 2000), it is difficult to make recommendations on optimal lobster juvenile density to avoid intraspecific competition and predation, but the new knowledge of predators on newly released lobster juveniles can be used to refine release techniques.

Even if the release techniques used so far were crude and based on guessing more than on fact, recaptures of both winter- and summer-released lobsters do occur regularly today. Relatively high numbers of released lobsters are landed compared with wild lobsters in the commercial lobster fishery of Kvitsøy 3–10 years after the release (van der Meeren and Næss 1991; Tveite and Grimsen 1995; Agnalt et al. 1999) but not enough to make it economically feasible. As long as the production price is more than 1£ per released lobster juvenile, loss of juvenile lobsters must be minimised to make the releases economically viable (Borthen et al. 1999).

Based on the present results and previous experience from lobster releases in Norway, the recommendations for when and where to release juvenile lobsters are as follows.

(*i*) Make a faunal investigation of the chosen release location by monitoring the fish and crustacean species in the area a full year prior to release. This should be done to be able to conduct the releases at the time of year for release when seasonally active predators are not present. In Nordic waters, the releases should be carried out in late winter – early spring to avoid loss due to the summer-active Labridae.

(*ii*) Release the juvenile lobsters on a substratum that offers sufficient shelter. Rocky bottoms are expected to be suitable, as long as they offer many crevices. Even if the diversity of other crustaceans and small fish is high in such habitats, the alternative sandy bottoms with no shelters have been found to result in higher loss to predators.

(*iii*) Predator-deterrent actions should not harm existing commercially and ecologically important species in the area to avoid conflicts with local fisheries and the possibility of disruption of the ecosystem supporting the commercial stocks.

Until more knowledge on the ecology of *H. gammarus* is available, the recommendations for where and when to release juvenile lobsters must be given with precautions, and further studies will be needed to confirm or refine the recommendations suggested.

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Habitat selection by juvenile coho salmon in response to food and woody debris manipulations in suburban and rural stream sections

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Abstract: This study explored the effects of food and woody debris manipulations on the summer distribution of juvenile coho salmon (*Oncorhynchus kisutch*) in small suburban streams. To examine fish responses to these factors, three different experiments were carried out in modified sections of two streams. The results showed that the distribution of juvenile coho salmon in a stream section was primarily controlled by the availability and distribution of food among pools and by the presence and density of woody debris. Food, however, played a dominant role because the foraging quality of a pool not only affected the density of fish in it but also the response of those fish towards instream debris. In food-rich stream sections, low proportions of juvenile coho salmon occupied pools with dense woody debris in the spring, which changed towards late summer. In contrast, in food-poor reaches, high proportions of fish were found in pools with abundant debris in the spring. Pools that combined abundant food with sparse woody debris were the most favoured by the fish. It is important that salmonid habitat enhancement projects consider that open foraging areas interspersed with woody debris characterize the type of summer habitat that juvenile coho salmon prefer.

Résumé : La présente étude examine les effets de la manipulation des aliments et des débris ligneux sur la distribution d'été du saumon coho (*Oncorhynchus kisutch*) juvénile dans les petits cours d'eau suburbains. Pour étudier la réaction des poissons à ces facteurs, trois différentes expériences ont été effectuées dans des tronçons modifiés de deux cours d'eau. Les résultats ont montré que la distribution du coho juvénile dans un tronçon était principalement régie par la disponibilité et la distribution de la nourriture dans les fosses ainsi que par la présence et la densité des débris ligneux. Le facteur dominant était cependant la nourriture, car la qualité de l'alimentation dans une fosse influe non seulement sur la densité de poissons, mais également sur la réaction des poissons face aux débris ligneux du cours d'eau. Dans les tronçons riches en aliments, de faibles proportions de cohos juvéniles occupaient les fosses très denses en débris ligneux au printemps, situation qui allait changer vers la fin de l'été. Au contraire, dans les tronçons pauvres en aliments, de fortes proportions de poissons ont été trouvées dans les fosses ayant d'abondants débris au printemps. Les fosses qui présentaient à la fois beaucoup d'aliments et peu de débris ligneux étaient les plus appréciées par les poissons. Il est donc important que les projets de mise en valeur de l'habitat des salmonidés tiennent compte du fait que les aires d'alimentation découvertes parsemées de débris ligneux caractérisent le type d'habitat d'été que le saumon coho juvénile préfère.

[Traduit par la Rédaction]

Introduction

In the Pacific Northwest, coho salmon (*Oncorhynchus kisutch*) find prime spawning and nursery habitat in small low-gradient coastal streams, with a large proportion of marginal areas, slow-flowing waters, and relatively equal proportions of alternating pools and riffles (Sandercock 1991). Juvenile coho salmon prefer pools to riffles and use instream structures, such as rocks and logs, as water current shelters to minimize the energy costs associated with maintaining position in the stream while feeding on drifting food (Mundie 1969; Fausch 1993). Logs (large woody debris (LWD)) increase pool frequency in both low- and moderate-slope stream reaches (Montgomery et al. 1995; Beechie and Sibley 1997), augment retention of sediments and particulate organic matter (Bilby 1981), and slow down the downstream migration rate of gravel (Montgomery et al. 1996).

Instream woody debris, both large and brushy or fine (FWD), may increase not only the availability of fish food (Nielsen 1992) but also the structural complexity of fish habitat. Instream structures make fish less visible to predators (Helfman 1981), provide physical refuge from predation (Reinhardt and Healey 1997), and increase intraspecific visual isolation among competitors (Dolloff 1986). Visual isolation reduces aggressive interactions among competitors and may therefore increase the number of young fish occupying a given area (Bugert et al. 1991`; Fausch 1993).

The topographic, hydrologic, and climatic conditions that make certain watersheds suitable for coho salmon are also well suited for human settlement. As a result, coho salmon nursery habitat in many watersheds of southwestern British Columbia has been affected by both agriculture (Birtwell et al.1988) and urban development (Slaney et al. 1996). In those systems, loss of instream woody debris, through both direct removal and reduced recruitment, is perhaps one of the most pervasive alterations to salmonid habitat (Booth

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1991). Managers have been trying to reverse this situation largely by means of instream debris placement projects. As a result, most habitat restoration projects are limited to the artificial installation of instream woody debris. Development of effective and efficient rehabilitation protocols depends in part on a clear understanding of how fish use such structures. However, there is no consensus in the literature on whether woody debris availability makes a stream reach more or less attractive to juvenile coho salmon during spring and summer. While some studies have found positive correlations between juvenile coho salmon and instream cover abundance (Dolloff 1986; Dolloff and Reeves 1990; Shirvell 1990), others have reported that the fish preferred to occupy open areas and showed no association with cover (Bugert and Bjornn 1991; Bugert et al. 1991; Spalding et al. 1995). Such contradictory results may be due to (*i*) the nature of the studies (i.e., descriptive versus experimental work), (*ii*) the type of system or habitat considered (i.e., small streams, artificial laboratory channels, or the mainstem of the river), (*iii*) the time of the year (early versus late summer), and (*iv*) the spatial scales considered (i.e., 40-m-long stream sections versus 1-m-long pools). Thus, the value of woody debris placement as a summer habitat enhancement technique remains somewhat controversial.

The objectives of this study were, first, to investigate the spatial distribution of juvenile coho in response to woody debris and food manipulations in stream habitat that has been altered by suburban residential development and farming activities and, second, to determine whether such responses change over time. To examine these responses, three different experiments were carried out in modified sections of two streams and were repeated throughout the summer. Two of these experiments (Experiments 1 and 3) evaluated the effects of woody debris and food on the spatial distribution of fish by manipulating woody debris and food abundance in the pools of an experimental stream section. The other experiment (Experiment 2) examined whether the spatial distribution of the fish was affected by woody debris density or type (LWD versus FWD) by placing logs in some stream pools and bundles of fine brushy debris in other pools.

Materials and methods

Study sites

Two of the three experiments were carried out in Spring Creek, Chilliwack, B.C., and the third one in Coghlan Creek, Langley, B.C. (Fig. 1). Both creeks run through agricultural land with interspersed residential areas and are characteristic of the type of habitat that wild juvenile coho salmon find on the rural–urban fringe of the Pacific Northwest.

Spring Creek originates at the base of Isar Mountain on the International Ridge of the Skagit Mountains and flows into Cultus Lake. The upper half of the stream, which runs through a golf course, has been cleared of riparian vegetation and is used by cutthroat trout (*Oncorhynchus clarki*). In contrast, the lower half, which is well shaded by the surrounding vegetation and has abundant overhanging cover and a mixed substrate of gravel and sand, is used by both cutthroat trout and chum salmon (*Oncothynchus keta*). Chum salmon spawn in early winter and their fry leave shortly after emergence, between March and April. Spring Creek had a coho salmon run that disappeared several years ago (D. Barnes, Cultus Lake Salmon Research Laboratory, 4222 Columbia Valley Highway, Cultus Lake, BC V2R 5B6, Canada, personal communication). The Canadian Department of Fisheries and Oceans presently stocks hatchery coho salmon fry into the lower half of the creek each spring. However, no adult coho salmon returns have been observed.

Water flow in Spring Creek remained relatively constant throughout the summer at 0.04 $m^3 \cdot s^{-1}$. Water temperature at the head spring was constantly 9°C but increased downstream. Daily water temperatures entering the experimental reach ranged from 12.3°C in May mornings to 22.9°C in August evenings.

The lower half of Spring Creek has a stairway-like aspect due to a series of low wooden weirs placed 20–50 m apart. The weirs create a series of waterfalls (20–40 cm high) that are separated by long pool/glide-like sections. The first glide immediately downstream from the golf course was used to build the experimental arena upstream from where the Department of Fisheries and Oceans had released coho salmon fry. This stream section was 20 m long and 4.5 m wide, and its upstream and downstream limits were set by 40-cm-high waterfalls. A net placed across the upstream waterfall prevented any of the fish used in the experiments from leaving this section. The downstream waterfall entered a fish trap box, which retained any fish trying to emigrate from the experimental section and blocked the upstream movement of other fish. The entire experimental section was longitudinally divided in half with a 2-mm-mesh fence to form two adjacent channels. Four pools and three riffles were constructed in each channel using large gravel and cobble. The pools were all similar, with a mean length of 4.1 m and a mean width of 2.0 m (mean area = 8.26 m^2). Their maximum depth was 0.5 m and their mean depth was 0.32 m. The dividing fence was partly buried in the substrate and was sealed to prevent fish movement between the two channels.

The site was considered ideal for food manipulations because invertebrate drift from the upstream golf course reach was very low. This was corroborated by drift and benthic samples. Four midwater drift samples were taken upstream from the experimental section on June 21, July 12 and 26, and August 11 using 1-m-long drift nets (200-μm mesh) with 5 × 15 cm mouths. After 24 h in the stream, the nets had no aquatic invertebrates and only a few terrestrial ones (mean = 2.25 insects per sample from orders Coleoptera, Diptera, and Hemiptera). Four benthic samples were taken at the same site using a 929-cm^2 Surber sampler with a 200-μm-mesh net. Two of those samples were taken on July 12 and the other two on July 26. These samples contained a few caddisfly (Trichoptera) larvae (mean = 1.62 larvae per sample) and a total of two worms (Annelida).

Coghlan Creek, the main tributary of the Salmon River, sustains a large coho salmon run and is also used by steelhead (*Oncorhynchus mykiss*) and cutthroat trout. Three sites near its headwaters were used as replicates for one of the experiments. In the summer of 1994, these sites had a mean flow of 0.06 $m^3 \cdot s^{-1}$ and their temperature ranged from 12.9 to 16.6°C (mean = 14.8°C). Two of the experimental sites, B and C, were 40 m apart in a shallow treeless ravine and lacked cover. The downstream site, C, was 19.5 m long and an average of 0.24 m deep and 3.4 m wide. The upstream site, B, was 18.6 m long and an average of 0.19 m deep and 2 m wide. A third site, A, was about 1 km further upstream. This site was 24.4 m long and averaged 0.35 m deep and 2 m wide. It was partially shaded by surrounding trees but did not have any instream cover. All three sites were glides with well-defined riffles at both ends. Three riffles of gravel and rocks about 1 m long and 0.03 m deep were constructed within each glide to create four pools of approximately equal size.

Experimental subjects

The fish used in Coghlan Creek were wild coho salmon from that creek, while in the Spring Creek experiments, coho salmon fry

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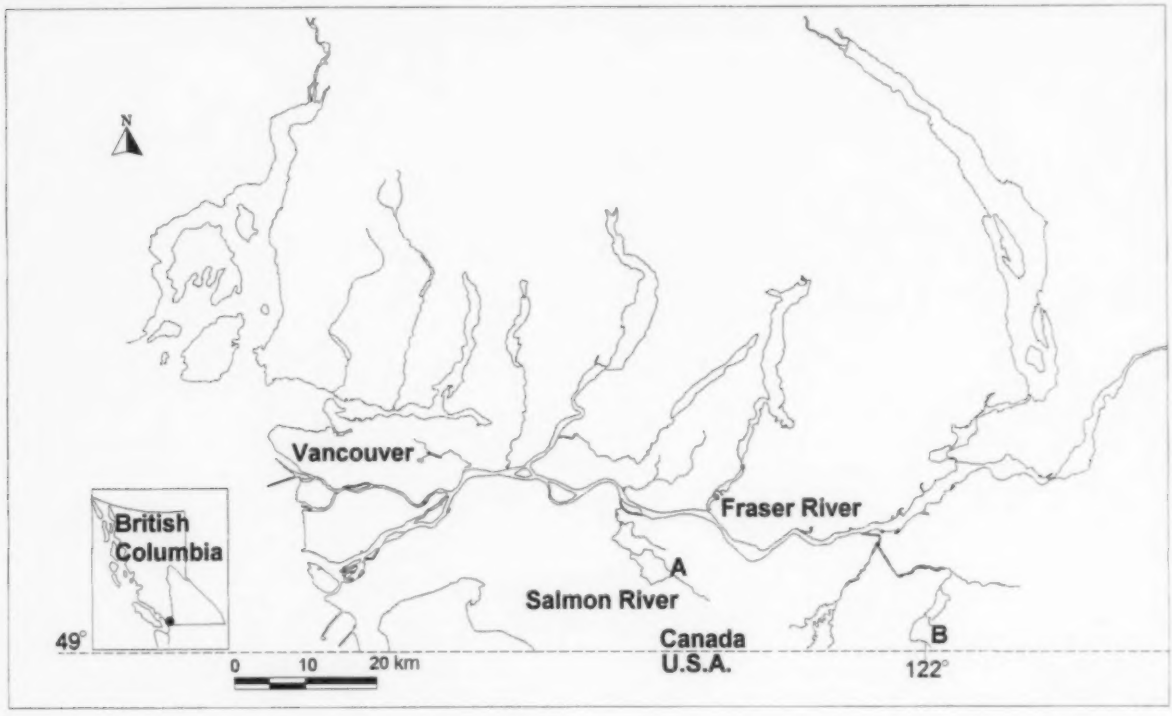
Fig. 1. Locations of Coghlan Creek (A, within the Salmon River watershed) and of Spring Creek (B, within the Vedder–Chilliwack system) in the lower Fraser Valley.



from the Chilliwack River Hatchery were used. These fish were kept in an outdoor holding channel (0.9 m wide, 0.4 m deep, and 17.4 m long) at the Cultus Lake Research Laboratory (Department of Fisheries and Oceans) from April 1 until they were used for the experiments. During the holding period, handling was kept to a minimum. Food (ground freeze-dried shrimp (*Euphasia pacifica*)) was delivered into the holding channel by means of automatic feeders placed 3 m apart from each other. This created a low but regular prey input rate intended to resemble invertebrates falling from the overhanging vegetation. Fish were exposed to avian predators because of the uncovered holding channel. Predation shelters were provided by bundles of two dozen 0.5-m-long twigs placed at 1.5-m intervals in the channel, and most fish were observed to make use of them.

All fish used in these experiments were cold branded using brass-made brands cooled in a mix of acetone and dry ice (see Bryant et al. 1990). For the purpose of this study, fish marking was only used to distinguish recaptured experimental individuals from potential immigrants to the experimental sites. This was particularly relevant for the Spring Creek experimental section.

During the experimental trials, fish were exposed to predation. American dippers (*Cinclus mexicanus*), green-backed herons (*Butorides striatus*), and kingfishers (*Ceryle alcyon*) were observed at all locations. Cutthroat trout of a size that could predate upon juvenile coho salmon were found at the three Coghlan Creek sites but were excluded from the Spring Creek experimental section.

Experiment 1

This experiment was carried out in the Spring Creek split channel section and tested the response of fish to woody debris presence and density under high- and low-food channel conditions. It examined whether (*i*) juvenile coho salmon distribution among pools is affected by woody debris availability and density, (*ii*) food abundance (food versus no-food channels) alters fish response to woody debris and (*iii*) fish response to woody debris changes over the summer.

Woody debris treatment consisted of tree branches grouped in bundles. These bundles resembled some of the FWD commonly present in coho salmon nursery habitat. However, they represented a simple test model, since they could not simulate all the parameters of niche separation and stability of spatial definition provided by some of the naturally occurring debris. Each bundle was made of eight red alder (*Alnus rubra*) branches (0.5–1.5 cm thick and 1.2–1.5 m long, with numerous offshoots each) combined with a branch of Douglas-fir (*Pseudotsuga menziesii*) and one of western redcedar (*Thuja plicata*) 1–1.2 m long, both with foliage intact. All branches were tied together and anchored with rocks so they would not drift away. The sunken alder branches provided fish with instream cover, and the fan-shaped coniferous branches that spread on the water surface served as overhead cover.

Freeze-dried shrimp was used as food treatment and was delivered by means of automatic belt conveyor feeders placed on the channel riffles. For this experiment, food treatment was randomly assigned to only one of the two channels before the beginning of each trial. In the treated channel, each pool received 7.5 g of freeze-dried shrimp per day (approximately a rate of 15% of biomass of released fish). In both channels, all pools were treated with one of three different amounts of debris: none, sparse FWD (one bundle of branches), or dense FWD (three bundles). Before releasing fish in the channels and starting a new trial, debris treatments were randomly assigned to pools (one of them was repeated, at random, because there were four pools in each channel). The sparse FWD treatment introduced a low density of cover that could offer predation refuge and visual isolation from competitors but, at

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the same time, left a large proportion of the pool unobstructed. In contrast, cover occupied almost the entire pool in the dense FWD treatment, leaving no open foraging areas.

Each fish was only used in one trial. At the beginning of each trial, equal numbers of randomly chosen fish were released into the Spring Creek's channels and allowed 72 h to explore and redistribute themselves among pools. At the end of each 72-h-long trial, fish were removed from the pools using a pole seine until none were caught in two consecutive passes. Thereafter, any fish remaining in the pools were captured by electrofishing. To prevent fish from switching pools during seine passes, stop nets were placed on the riffles and at the downstream waterfall.

In the early summer trials (May 24 to June 27), 50 juvenile coho salmon (mean length = 4.32 cm, SD = 0.425, n = 200; mean weight = 1.03, SD = 0.318, n = 200) were released into each experimental pool per trial (total = 200 fish in each experimental channel). The number of fry released into each pool was sufficient to create a very high initial density (6 fish·m^{-2}), similar to early summer densities reported for some highly productive coho salmon streams in this part of the province (Ptolomy 1993). Under these conditions, a large proportion of juvenile coho salmon moved downstream and entered the fish trap as expected (see Chapman 1962). Ten trials were carried out for each food treatment. For the late summer trials (August 18 to September 9), the number of juvenile coho salmon released into the channels was reduced to 25. Because the fish were larger (mean length = 5.71 cm, SD = 0.467, n = 100; mean weight = 2.06 g, SD = 0.532, n = 100), this number gave a similar biomass per pool to that in the earlier trials (about 6.15 g·m^{-2}). Due to time constraints, the number of trials in this case was reduced to seven for each food treatment.

The design of this experiment corresponded to a split-plot factorial. Food was the main plot factor (with two levels: food versus no food) and woody debris was the subplot factor (with three levels: none, sparse, and dense). The experiment was replicated over time. Because the waterfall at the upper end of both channels seemed to attract fish into the upstream pools in the absence of treatments (see control experiment), analysis of covariance (ANCOVA) was used to separate the effect of this factor from those of food and woody debris on fish distribution. Two covariables were used: "distance" (between the centre of each pool and the upstream end waterfall) and "distance^2," because the control experiment revealed that a quadratic regression provided the best "fit" for predicting fish abundance based on pool distance to upstream waterfall.

Experiment 2

This experiment was carried out in the three Coghlan Creek sites and examined whether (*i*) juvenile coho salmon distribute among stream pools in response to woody debris density and type (LWD versus FWD) and (*ii*) fish response to woody debris changes over time.

Before beginning experimental trials, pools in each of the three sites were closed with nets (5-mm mesh) and all fish were removed by pole seining and electroshocking. Fish were counted and sorted by species into separate holding buckets. Besides coho salmon, three other species of fish were found: rainbow/steelhead trout, cutthroat trout, and threespine stickleback (*Gasterosteus aculeatus*). After removing the fish, different debris treatments were randomly assigned to pools.

Each of the four pools built at each site received one of the following debris treatments: no debris, sparse LWD, sparse FWD, or dense FWD. The LWD treatment consisted of two logs (diameter of at least 20 cm) forming a "Y" in the pool. The logs were anchored to the stream banks with nylon rope and were submerged under the weight of three boulders. Pools treated with sparse FWD received three bundles of branches, which were anchored to bank rocks with nylon rope. The bundles were made of eight red alder branches and one fan-shaped western redcedar branch, similar in dimensions to those used in Spring Creek. Pools treated with dense FWD were entirely covered with nine bundles.

After debris placement, all fish but juvenile coho salmon were returned to the pools from which they had been caught. At each site, juvenile coho salmon were divided into four equal groups to create equal densities in all pools. Fish were individually measured (June: mean length = 4.43 cm, SD = 0.578, n = 100; mean weight = 1.05, SD = 0.457, n = 100). Afterwards, each group of fish was randomly assigned to and released into one of the four pools. From 75 to 120 fish were released per pool, depending on the number of fish caught at each experimental site. Trials began in June, and fish at each site were sampled again in July and August (July: mean length = 4.78 cm, SD = 0.676, n = 100; mean weight = 1.39, SD = 0.655, n = 100; August: mean length = 4.93 cm, SD = 0.547, n = 100; mean weight = 1.36, SD = 0.486, n = 100). During each of these sampling times, cover treatments were randomly redistributed among pools before coho salmon were released back into the pool in which they were found. Thus, although the type of cover that coho salmon found in their pools may have changed, each individual found itself in the same pool that it had occupied just before sampling began.

This experiment had a randomized block design (blocks were monthly replications) with two factors (debris and site) and its data were examined using analysis of variance (ANOVA) (Hicks 1993). The effect of debris on fish distributions was also tested separately for each month using single-factor ANOVA.

Experiment 3

This experiment was carried out in the Spring Creek split channel and examined whether woody debris or food alone or the combination of both factors controls the distribution of juvenile coho salmon among pools.

Two treatments were used in it, food and FWD, and each treatment had two levels: food versus no food and dense FWD versus no debris. Each one of the four possible treatment combinations was randomly assigned to one of the four pools in each channel.

Floating shrimp were prevented from drifting between pools by means of small-mesh screens placed at the tails of the pools. The screens sat on wire legs above the substrate, leaving enough space for fish to swim underneath. Their mesh was cleaned every 8–10 h to keep it from clogging. However, although no evidence of overflow was ever found, the possibility of food drifting among pools remained. Twenty-five fish were released into each pool at the beginning of each trial. Eleven trials were completed between July 22 and August 11.

This experiment, with its two factors (debris and cover) and a randomized block (trial) design, was analyzed using ANCOVA. As in Experiment 1, distance and distance^2 to upstream end waterfall were the covariables used to adjust for the effect that the waterfall had on the distribution of fish. In contrast with Experiment 1, which could not test the effect of the interaction among treatments because woody debris was a "subtreatment " (subplot factor) applied to pools within food-rich or food-poor channels, this experiment examined the main effects of both woody debris and food as well as their interaction on fish distribution.

Control experiments

In the experimental section of Spring Creek, pool distance from upstream waterfall seemed to affect pool choice by fish. Therefore, a control experiment was used to examine how juvenile coho salmon distributed among pools in the absence of any experimental treatments. Fifty juvenile coho salmon were released per pool and their distribution was recorded 72 h later. This experiment was replicated 10 times. Six trials were carried out between July 1 and 11 and four trials between August 12 and 18.

This was a single-factor experiment with a randomized block (trial) design and was analyzed using ANOVA. The main factor

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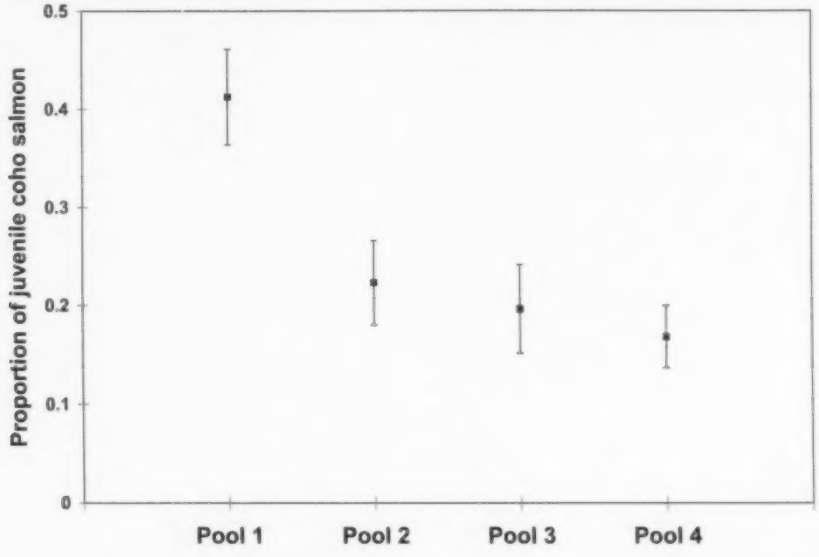
Fig. 2. Proportional distribution of juvenile coho salmon among control experimental channel pools. Squares are mean values and vertical lines represent ±2 SE.



was pool distance to upstream end waterfall. Based on Fig. 2, a quadratic model was chosen as a first approximation to predict "fish distribution" from pool distance. The ANOVA was performed on a model that partitioned pool distance into linear and quadratic components and the interaction term into distance by trial and distance^2 by trial. Its results confirmed that pool distance ($F = 44.14$, df = 1, $p < 0.001$) and pool distance^2 ($F = 9.93$, df = 1, $p = 0.011$) from upstream end waterfall affected fish distribution among pools. Therefore, both pool distance and pool distance^2 were used as covariables in the analyses of Experiments 1 and 3.

Because Chilliwack River hatchery fish were used in the Spring Creek experiments and wild fish were used in the Coghlan Creek experiment, another control test was carried out to determine whether juvenile coho salmon of those two stocks showed different habitat preferences in a common experimental channel.

An artificial outdoor channel at the Cultus Lake Research Laboratory was used for this test. The experimental channel was shaped as a "B" with a straight 10-m-long, 0.9-m-wide, and 0.05-m-deep riffle and two curved side pools (3 m long, 0.9 m wide, and 0.5 m deep) connected to it. The channel received Cultus Lake water and its temperature was maintained at 13°C. Pool water velocity was 1 cm·s^{-1}, and riffle water velocity was 20 cm·s^{-1}. A dense FWD bundle (of the type described earlier) was randomly assigned to one of the two pools at the beginning of each trial. Groups of 24 fish, either all hatchery or all wild in origin, were released into the pools (12 in each one) at the beginning of each trial and their final distribution recorded 72 h later. Every evening, from 7 to 9 p.m., 5 g of shrimp was delivered into each pool by means of automatic feeders. Ten replicates were obtained, five with each fish type, between August 9 and September 15.

The numbers of wild and hatchery fish found in each habitat type at the end of this experiment's trials were compared using a t test ($p = 0.05$), and no difference in pool choice was observed between these two types of fish in the artificial channel ($t = 1.45$, df = 8, $p = 0.176$).

Data analyses common to all experiments

All statistical analyses were carried out using SAS systems software (SAS Institute Inc. 1988).

In all experiments, except the control comparison between hatchery and wild fish, data were expressed as proportions (number of fish present in individual pool/total number of fish present in the channel) to compensate for different fish densities in early and late summer trials and among experimental sites. The Shapiro and Wilk test (W) (Zar 1984) was used to test departures from normality. Whenever interactions between factors were significant, the means of one of the factors were compared separately for each level of the other factor using Tukey's studentized range test, which controls for Type I experimentwise error rate (Zar 1984).

Results

The Shapiro and Wilk test indicated that data were normally distributed in all cases, and therefore, no transformations were applied.

Fish immigration into Spring Creek's experimental section was negligible and all fish caught at the end of each trial were those that had been released at the beginning of those same trials. Only two unmarked fish were found in this section during any of the experimental or control trials. In Coghlan Creek, fish marks showed that by late summer, 68.7% of the fish were occupying the same creek sections were they had been found in early June (Site A = 61.5%, Site B = 73.5%, and Site C = 71.2%).

Experiment 1

Choice of pool by young coho salmon was not significantly affected by either FWD ($F = 0.48$, df = 2, $p = 0.626$) or food treatments ($F = 0.03$, df = 1, $p = 0.865$) in early summer. In contrast, the interaction between both treatments had a significant effect on the distribution of the fish ($F = 3.37$, df = 2, $p = 0.050$), suggesting that fish responded in a different manner to woody debris presence and density depending on whether food was abundant in the channel or not. Higher proportions of fish occupied pools without FWD than pools with dense FWD in trials with food treatment; but

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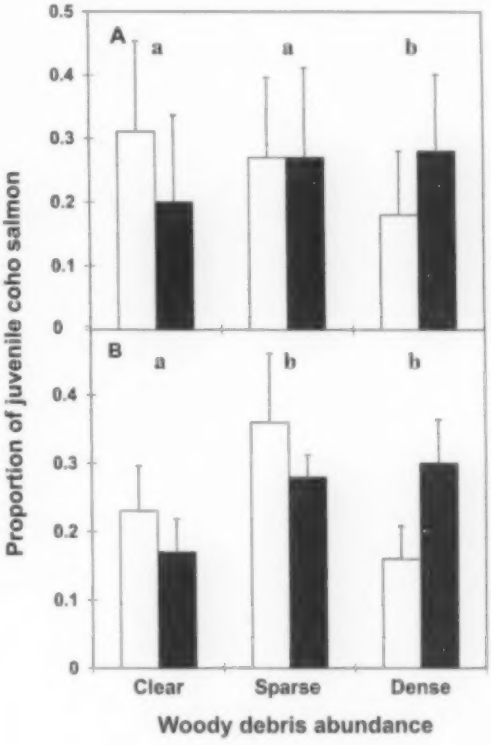
without food, higher proportions of fish preferred pools with dense FWD to clear pools. The proportion of juvenile coho salmon found in pools with sparse FWD was the same under both food treatments (Fig. 3A).

In the late summer trials, woody debris also had a significant effect on fish distribution ($F = 4.21$, df = 2, $p = 0.037$), whereas food did not seem to influence fish behaviour ($F = 0.001$, df = 1, $p = 0.976$). The interaction between debris and food also had a marginally significant effect on fish distribution ($F = 3.46$, df = 2, $p = 0.051$) (Fig. 3B), but with an inverse trend in the proportion of fish choosing pools with either no debris or with dense FWD depending on food treatment.

Albeit always influenced by food abundance, woody debris density had a stronger effect on fish distribution in late summer than in early summer (Tukey's studentized range test; early summer trials: pools without debris and pools with sparse FWD were grouped together and pools with dense FWD were classified by themselves; late summer trials: pools without debris formed their own group and pools with FWD debris, both sparse and dense, were grouped together). This difference, however, seemed quantitative rather than qualitative. The main distinction was that, independently of food treatment, there were lower proportions of fish in pools without woody debris in late summer than earlier in the season. Otherwise, fish distribution among pools in response to debris and food treatments followed similar trends in both early and late summer trials (Fig. 3).

Fig. 3. Proportional distribution of Spring Creek coho salmon fry among channel pools receiving one of the following treatments: sparse FWD, dense FWD, or no woody debris. Channel treatments were food (open bars) or no food (solid bars) in (A) early summer and (B) late summer. Bars represent mean values and vertical lines represent 2 SE. Treatments with the same lowercase letters were classified in the same group with Tukey's test ($\alpha = 0.05$).



Experiment 2

Combined data from all three Coghlan Creek sites for the months of June, July, and August showed that woody debris treatments had a significant effect on pool choice by juvenile coho salmon ($F = 5.09$, df = 3, $p = 0.044$). The interaction between debris and site was not significant ($F = 1.08$, df = 6, $p = 0.424$), indicating that fish response to debris treatments did not differ among experimental locations. The statistical model was expanded to include an estimate of the monthly replicate by woody debris interaction. This interaction was considered significant ($F = 3.09$, df = 6, $p = 0.0514$) and is supported by trends shown in Fig. 4.

Separate analyses by month showed changes in the effect of woody debris on fish pool selection. In June, debris did not affect fish distribution among pools ($F = 0.81$, df = 3, $p = 0.525$) whereas in July, it did have a significant effect on pool choice by fish ($F = 13.72$, df = 3, $p = 0.002$), with sparse FWD treated pools attracting a higher proportion of fish than the other pools (Tukey's studentized range test) (Fig. 4). In August, the effect of debris on fish distribution was also significant ($F = 8.67$, df = 3, $p = 0.007$) and fish were equally attracted to pools with sparse debris density regardless of debris type (Tukey's studentized range test combined sparse FWD and sparse LWD in one group and placed dense FWD and pools with no cover in another group) (Fig. 4).

Experiment 3

In this experiment, both food ($F = 21.82$, df = 1, $p < 0.001$) and the interaction between food and FWD ($F = 5.19$, df = 1, $p = 0.049$) had a strong effect on the distribution of juvenile coho salmon among pools. In contrast, FWD did not influence pool choice ($F = 1.14$, df = 1, $p = 0.311$). High proportions of young coho salmon were found in pools with food and low proportions in pools without food (Fig. 5), where Tukey's studentized range tests confirmed the difference between food and no-food treatments by classifying them separately. These results indicate that juvenile coho salmon responded primarily to food and that woody debris altered such response.

Discussion

In suburban and rural streams, the summer distribution of juvenile coho salmon in salmon-bearing reaches is primarily governed by the abundance of food and their distribution among pools, by the presence and density of instream woody debris, and by hydrological features such as small waterfalls. Pool foraging quality affects not only the number of juvenile coho salmon that occupy a pool, but also the response of those fish towards instream debris. In a food-rich environment, the association between coho salmon and instream woody debris is low in May and June but increases as the summer progresses. In mid- to late summer, the presence of low to medium densities of woody debris (either

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Fig. 4. Proportional distribution of Coghlan Creek coho salmon fry among pools that received one of the following treatments: no cover, sparse LWD, sparse FWD, or dense FWD. Bars represent mean values for three different experimental sites and vertical lines indicate 2 SE. Open bars, June; solid bars, July; stippled bars, August. Within each month, treatments with the same lowercase letters were classified in the same group with Tukey's test ($\alpha = 0.05$).

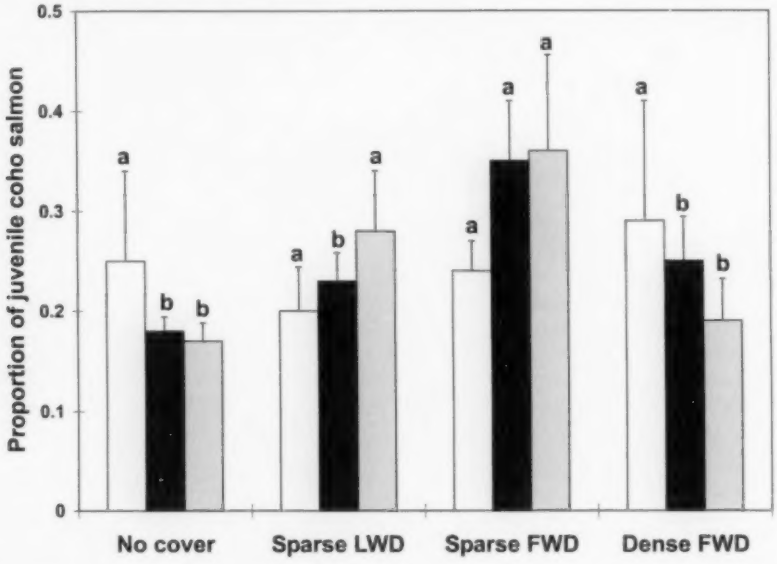
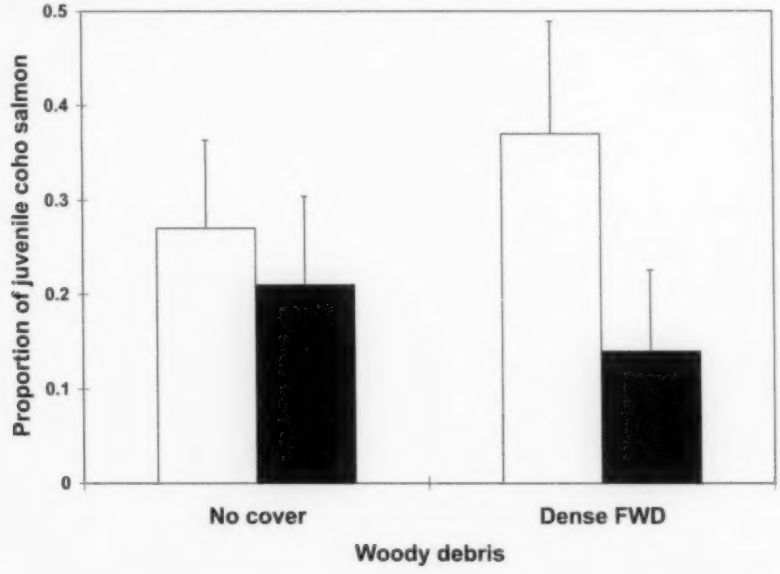


Fig. 5. Proportional distribution of Spring Creek coho salmon fry among pools receiving one of the following treatments: no cover and food, cover and food, no cover and no food, and cover and no food. Bars represent mean values and vertical lines represent 2 SE. Open bars, food; solid bars, no food.



large or fine) enhances the quality of stream pools as nursery habitat.

Food abundance alters the response of juvenile coho salmon to woody debris density, and this response changes over the summer months. Although fish responded to the presence of woody debris in different manners depending on food availability, pools with intermediate debris densities were chosen by relatively high proportions of individuals in most trials. Instead of always choosing pools with the highest density of debris to minimize predation risk, when food was abundant, most fish selected pools with either sparse FWD or no debris at all. This suggests that the young fish,

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when given the chance, tried to maximize their food intake rather than balance foraging opportunity with cover use. Gilliam and Fraser (1987) observed similar behaviour in creek chub (*Semotilus atromaculatus*) that spent a decreasing amount of time in a predation refuge (without food) as the food level increased in the unprotected environment. In the case of visual feeders, like coho salmon and other salmonids, it is likely that underwater structures may have a negative impact on their foraging efficiency that is strong enough to cause what appears to be risk-prone behaviour (Ware 1973).

The fact that in trials without food addition, the proportion of juvenile coho salmon found in pools with dense FWD was higher, largely at the expense of pools with no cover, further supports the idea that fish display risk-prone behaviour as long as it pays off (i.e., with higher food intake rate). Fish response to woody debris did not change markedly over time in the absence of food treatment. Most fish preferred pools with sparse or dense cover, both in early and in late summer. However, when food was added to the pools, fish response to FWD changed. In early summer, the young coho salmon preferred pools with sparse or no FWD, whereas in late summer, the majority of fish chose pools that offered sparse FWD. This difference in fish behaviour between May and August trials in Spring Creek could have been caused, at least in part, by the different length of holding channel residence experienced by the fish used in these trials. However, it is important to point out that wild fish in food-rich Coghlan Creek also showed an increased preference over time for pools with sparse woody debris, at the expense of pools without debris.

Woody debris type may influence fish pool selection. Juvenile coho salmon distributions in the experimental sites of Coghlan Creek were not affected by either debris density or debris type in June (early summer), but they were in July and August, when the young fish showed a preference for pools with sparse debris. In July, pools with sparse FWD attracted a greater number of fish than pools with sparse LWD, dense FWD, or no debris. By August, however, the fish did not seem to discriminate between debris types (fine versus large) as long as the pools offered both large open foraging areas and a low density of structures. Reasons for the brief preference for fine brushy cover over LWD are not immediately apparent but could have resulted from the experimental setup. For example, differences in invertebrate availability among microhabitats may have been induced by the type of debris.

Despite differences in fish origin (hatchery versus wild), stream productivity, and experimental setups, the observed distributions of fish among pools in response to woody debris treatment and their changes over time followed similar patterns in both creeks. The control test results also indicated comparable habitat preferences between fish from the two stocks used in this study.

The presence of food rather than cover seemed to be the preeminent factor determining juvenile coho salmon habitat preference. However, woody debris availability altered fish response to food. It is possible that woody debris affected the spatial distribution of coho salmon by hindering their ability to detect food (Giannico and Healey 1999), reducing aggressive interactions among competitors (Chapman 1962; Fausch 1993), and providing various risk–reward conditions (Dill and Fraser 1984).

Food predominance over cover in determining suitable salmonid nursery habitat has already been observed in other species. For example, Wilzbach (1985) found that food abundance was more important than cover in determining the abundance and microhabitat distribution of adult cutthroat trout in laboratory channels. This could explain why almost 70% of the coho salmon chose pools with food treatment. Among them, a slight majority preferred pools that also offered cover. The possibility that sparse cover would have been strongly preferred, as was observed in other experiments, also exists. Thirty percent of the fish occupied pools with scarce food for a variety of possible reasons (e.g., inadequate habitat sampling, displacement from more favourable sites by dominant individuals, avoidance of competition, and even the possibility that some shrimp could have drifted among pools, increasing the actual foraging value of the pools that did not receive food treatment). The fact that two thirds of these fish were in pools without cover suggests that many individuals gave priority to prey detection over predation-risk reduction (Reinhardt and Healey 1997). As Dill and Fraser (1984) observed, hungry coho salmon are prepared to take more risk and to continue foraging even when predators are sighted.

In the absence of food and debris, high proportions of fish moved into the upstream-end pool of each Spring Creek experimental channel. Such preference for pools with waterfalls could be attributed to (*i*) the cover generated by water turbulence and (or) (*ii*) the availability of fast-moving waters next to slow-moving waters, which offer the combination of foraging and resting patches that juvenile coho salmon seem to prefer (Fausch 1993).

These results may explain, at least in part, the conflicting reports in the literature about use of cover by coho salmon. Studies conducted in spring and early summer (Bugert and Bjornn 1991; Bugert et al. 1991; Spalding et al. 1995) did not find a preference for cover whereas those conducted later in the summer did (Dolloff 1986; Dolloff and Reeves 1990; Shirvell 1990). It is possible that this change in cover use over time can be induced, at least in part, by predation risk. Several studies have shown that juvenile coho salmon increase their use of cover after exposure to predation (Dill and Fraser 1984; Grand and Dill 1997; Reinhardt and Healey 1997). Furthermore, predation risk does not seem to elicit the same response in all individuals. Reinhardt and Healey (1997) observed that in the absence of predation, juvenile coho salmon distributed independently of cover, but after being exposured to a real or simulated predator, some individuals increased their use of cover whereas others changed their foraging strategies and moved away from bundles of FWD. In their study, the larger (presumably dominant) fish were more likely to seek cover (risk averse) in the presence of a predator; this created feeding opportunities in open areas for the smaller individuals that seemed prepared to risk exposure to predation. In this study, fish were exposed to wild predators in both the outdoor holding channel and the experimental stream sections. Therefore, it is possible that as size difference among individuals increased over the summer, different foraging strategies developed under predation risk, causing the observed seasonal changes in

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cover use. Unfortunately, this hypothesis could not be tested because neither the size nor the response to cover of individual fish was recorded in this study.

Increased preference for pools with woody debris is a behavioural change associated with the habitat shift that juvenile coho salmon undertake with the first fall freshets (Sandercock 1991). As water temperature falls below 4.5°C, the level of activity of coho salmon decreases markedly and they move closer to objects providing cover (Bustard and Narver 1975). By late August or early September, however, neither water flow nor water temperature had begun changing significantly to support the idea that fish had started to seek winter habitat in response to such environmental clues. If late summer reduction in invertebrates is what increases fish use of cover in preparation for less favourable winter conditions, only coho salmon in Coghlan Creek should have shown such behaviour. Fish response to woody debris in Spring Creek increased towards late summer, despite the fact that they were receiving the same food supplement as in early summer. The only environmental factor that changed in both experiments between early and late summer trials was the photoperiod, and it is possible that shorter days may increase fish association with cover in preparation for winter conditions (Bustard and Narver 1975). In addition, the larger body size of fish by the end of summer could account for increased risk aversion (Reinhardt and Healey 1997) and preference for pools with woody debris.

In many agricultural and suburban streams, where the natural supply of woody debris is limited, it is common practice to enhance salmonid habitat by adding LWD. When planning such projects, it is important to consider that high densities of woody debris in the stream channel can negatively affect juvenile coho salmon abundance just as its absence can. This study indicates that open foraging areas interspersed with woody debris (LWD and FWD) and small hydrological structures (waterfalls) characterize the summer habitat that juvenile coho salmon prefer. The artificial placement of woody debris may only increase salmonid densities in the treated sections due to fish immigration from other sections (Riley and Fausch 1995) and locally enhanced carrying capacity (Culp et al. 1996) but may not affect the size of the population in the entire stream during summer. However, fish winter survival, and therefore smolt production, may increase as a result of increased availabilty of instream woody debris (Quinn and Peterson 1996).

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Multiple lineages of the mitochondrial DNA introgression from *Pungitius pungitius* (L.) to *Pungitius tymensis* (Nikolsky)

H. Takahashi and K. Takata

Abstract: We applied mitochondrial DNA (mtDNA) restriction fragment length polymorphism analysis to trace the maternal lineages and compare the intrapopulation mtDNA variabilities of two ninespine stickleback species, *Pungitius pungitius* (L.) and *Pungitius tymensis* (Nikolsky), in which occasional natural hybridization was detected. mtDNA phylogenies constructed by neighbor-joining and Wagner parsimony methods resolved two divergent clades differing by an average nucleotide divergence of 5.94%. All *P. pungitius* mtDNA haplotypes fall within a single clade. In contrast, *P. tymensis* appeared paraphyletic; nine of the 13 mtDNA haplotypes found in *P. tymensis* were grouped within the *P. pungitius* clade, but the remaining four were of the other clade. The former nine mtDNA variants were very close to the sympatric *P. pungitius* mtDNA halotypes. These observations suggest that the nine *P. tymensis* haplotypes originated and introgressed from sympatric *P. pungitius* and that the introgressed mtDNA haplotypes have a polyphyletic origin in each region. The high incidences of the mtDNA introgression in northern and central Hokkaido may be due to a warmer climate than that of the other regions. The recipient *P. tymensis* populations had significantly higher mtDNA variation than those observed in the sympatric donor species populations, suggesting the sudden population growth during or after the mtDNA substitution processes.

Résumé : Nous avons appliqué l'analyse des polymorphismes de restriction de l'ADN mitochondrial (ADNmt) pour retracer les lignées maternelles et comparer les variabilités intrapopulation de l'ADNmt chez deux espèces d'épinoche à neuf épines, *Pungitius tymensis* (L.) et *P. Pungitius* (Nikolsky), chez qui on a observé à l'occasion une hybridation naturelle. La reconstitution de la phylogenèse de l'ADNmt par les méthodes du plus proche voisin et de la parcimonie de Wagner a fait ressortir l'existence de deux variantes différant en moyenne de 5,94% de divergence des nucléotides. Tous les haplotypes d'ADNmt de *P. pungitius* se retrouvent dans une seule variante. Par contre, *P. tymensis* semble paraphylétique; neuf des treize haplotypes d'ADNmt observés chez *P. tymensis* étaient regroupés dans la variante de *P. pungitius*, mais les quatre autres possédaient l'autre variante. Les neuf premiers variants d'ADNmt étaient très proches des ADNmt des *P. pungitius* sympatriques. Ces observations permettent de penser que les neuf haplotypes de *P. tymensis* proviennent des *P. pungitius* sympatriques, par introgression, et que les haplotypes d'ADNmt introgressés ont une origine polyphylétique dans chaque région. La forte incidence d'introgression de l'ADNmt dans le nord et le centre d'Hokkaïdo peut être due au climat plus doux que dans les autres régions. Les populations réceptrices de *P. tymensis* présentaient une variation de l'ADNmt nettement plus élevée que celle qui a été observée chez les populations sympatriques des espèces donneuses, ce qui semble indiquer qu'une croissance soudaine de la population est survenue pendant ou après les processus de substitution de l'ADNmt.

[Traduit par la Rédaction]

Introduction

In recent years, there has been a renewal of interest in the evolutionary importance of natural hybridization, especially as a source of new genetic variability within taxa (reviewed in Arnold 1997). Although there is increasing evidence for occurrence of genetic introgression among fish species (e.g., Bernatchez et al. 1995; Chow and Kishino 1995), little is known about the effect of interspecific introgression on the genetic structure of recipient populations. In a classic review of fish hybridization, Hubbs (1955) stated that the frequency of hybridization may be increased in labile environments, leading to reduction of spawning areas or numerical minority of one species relative to a related form. Such rare forms are more likely to hybridize, potentially increasing levels of introgression from the common into the rare form. Recently, comparison of the patterns of genetic introgression among multiple sympatric pairs of species has provided a new research direction to understand the historical effects in evolution of reproductive isolation (Dowling et al. 1997). This method can also be applied to examine the changes in genetic structure of introgressed populations; however, no studies have been done using this method for this purpose.

Since genetic introgression may be detected by incongruity in the phylogenetic histories of various genetic markers (Arnold 1997), it is nessessary to use a sensitive marker for

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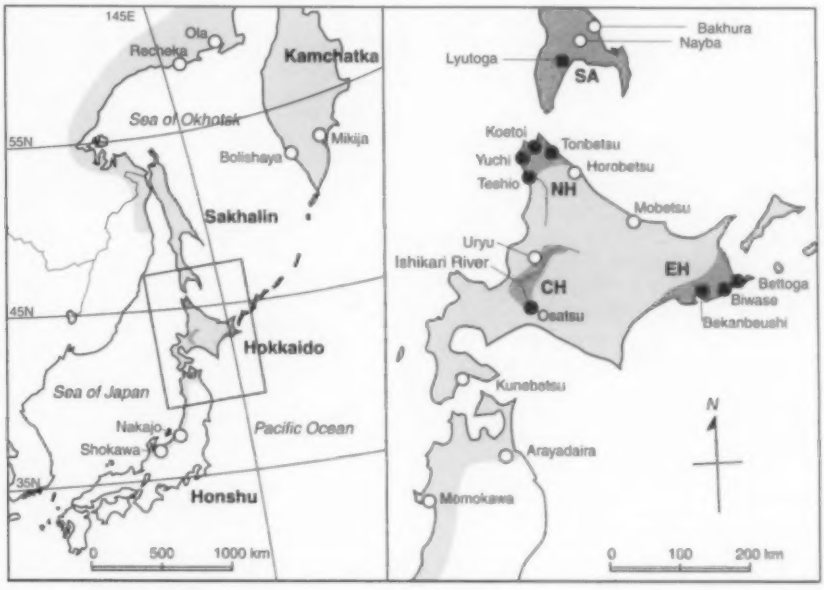
Fig. 1. Distributions of *P. pungitius* and *P. tymensis* in the northern Far East. The shaded and dotted areas on the maps indicate the distributions of allopatric *P. pungitius* populations and the sympatric regions of the two species, respectively. Solid circles indicate sampling sites where both species were collected, and open circles indicate sampling sites where either *P. pungitius* or *P. tymensis* were collected in the present study. Sympatric regions: SA, Sakhalin; NH, northern Hokkaido; EH, eastern Hokkaido; CH, central Hokkaido.



reconstructing gene genealogy. Mitochondrial DNA (mtDNA) is a useful marker because it is an independently inherited unit of DNA, generally considered to be nearly selectively neutral (but see Ballard and Kreitman 1995), and analysis of a simple model shows that the transmission of mtDNA can easily occur across species boundaries unless there is extremely strong selection against hybrid females (Takahata and Slatkin 1984). Furthermore, mtDNA data provide information not only on occurrence of the introgressive hybridization, but also on past population sizes from the genetic structure of a population (e.g., Ovenden and White 1990; Merilä et al. 1997). In particular, mtDNA variability is expected to be profoundly affected by historical demographic change compared with nuclear DNA because mtDNA is haploid and inherited primarily along matrilines in vertebrate species (Takahata 1986).

Pungitius pungitius (L.) and *Pungitius tymensis* (Nikolsky) are small freshwater fish distributed in the northern Far East. *Pungitius pungitius* is widely distributed throughout the Northern Hemisphere (Wootton 1976) and continuously distributed in the northern Far East (Takata et al. 1984). On the other hand, *P. tymensis* is isolated in the following four regions: southern Sakhalin, northern and eastern Hokkaido, and the Ishikari River basin in central Hokkaido (see Fig. 1) (Takata et al. 1984). In most of these regions, *P. pungitius* is sympatric with *P. tymensis*. When they inhabit the same river, *P. tymensis* tends to occupy the upper reaches of the stream (Kobayashi 1957; Takata et al. 1984).

Niwa (1987) found significant differences in gene frequencies at four allozymic loci of *P. pungitius* and *P. tymensis* and suggested that they are reproductively isolated even when they coexist. Previous allozymic studies revealed the absence of population subdivision within both *P. pungitius* and *P. tymensis* in Hokkaido (Niwa 1987; Takata 1987). However, natural hybridization between the two species was detected in several rivers in Hokkaido, as evidenced by the appearance of the intermediate morphs (Kobayashi 1957, 1959) and of an interspecific combination of alleles of a nuclear locus (Niwa 1987). Furthermore, allozyme phenotypes indicated the presence of second-generation hybrids of and (or) back-crosses to both species (Niwa 1987). Artifical hybridization demonstrated that F1 hybrids were reciprocally fertile (Kobayashi 1959). These results potentially satisfy a qualification of Takahata and Slatkin's (1984) model and suggest the potential for mtDNA introgression between the two species.

The purpose of the present study was to test the prediction that introgressive hybridization occasionally increases the genetic variability in the recipient population. First, we traced the maternal genealogies of the two species by restriction fragment length polymorphism analysis of the entire mtDNA to elucidate the occurrence, process, and directionality of ancient hybridization and introgression. The four sympatric regions of *P. pungitius* and *P. tymensis* were investigated to compare the introgression patterns among the regions, and allopatric populations of *P. pungitius* were added to the investigation to ascertain their original mtDNA lineage. Second, we analyzed mtDNA genetic variation within populations to compare the levels among individuals carrying the introgressed and nonintrogressed and their donor species. The prediction was tested by ascertaining

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whether or not the genetic variation in introgressed populations is higher than that in nonintrogressed populations. Furthermore, comparisons of mtDNA genetic variation in nonintrogressed populations among four sympatric regions were made to determine the cause of occurrence of introgressive hybridization.

Materials and methods

Sample collection

Twenty-two populations of *P. pungitius* and (or) *P. tymensis* were collected in the northern part of Japan and the Far East of Russia (Fig. 1). Initial sampling efforts were focused on the four sympatric regions of these species: southern Sakhalin, northern and eastern Hokkaido, and the Ishikari River basin in central Hokkaido. Sampling was extended to allopatric populations to infer the authentic mtDNA lineages of each species. All samples were quickly frozen on dry ice or in liquid nitrogen and kept –85°C until they were analyzed.

Specimens were identified as *P. pungitius* or *P. tymensis* based on morphological characteristics following Lindberg and Legeza (1969). In addition, the number of dorsal spines by which the two species are clearly distinguishable (Takata et al. 1984) was used to separate the species. Since Takata (1987) and Niwa (1987) also confirmed that the morphological identification of these species and their hybrids was consistent with this identification method based on diagnostic allozyme loci, the species were identified only by morphological characteristics.

Preparation of probes

Closed circular mtDNA was isolated from mature ova of *P. pungitius* females collected in the Kunebetsu River using CsCl-gradient centrifugation (Lansman et al. 1981). mtDNA was digested into seven fragments with the restriction endonuclease *Hin*d III. Six of seven fragments (3.60, 3.50, 3.20, 1.70, 1.40, and 0.55 kilobase pairs (kbp)) were ligated into the *Hin*d III site of pUC19. The remaining fragment (2.35 kbp) was ligated into the *Hin*d III site of Bluescript KS ($M13^-$). Competent *Escherichia coli* strain DH5α (TaKaRa Co., Tokyo) was transformed with the ligated plasmids to amplify the plasmid DNA. Amplified plasmid DNA was purified using the QIA-prep plasmid DNA purification kit (QIAGEN Inc., Valencia). Each insert was labeled by digoxigenin using a nonradioactive DNA labeling and detection kit (Boehringer Mannhaim Biochemicals, Indianapolis, Ind.).

DNA preparation and restriction fragment length polymorphism analysis

Total DNA was extracted from muscle tissue of frozen samples in TUES–urea (10mM Tris–HCl, pH 7.5, 125 mM NaCl, 10 mM EDTA, pH 7.5, 0.5% SDS, and 4 M urea) (Asahida et al. 1996) along with proteinase K (100 $\mu g \cdot mL^{-1}$) and incubated at 50°C for 3 h. After incubation, total DNA was purified by standard phenol–chloroform extractions and ethanol precipitations (Sambrook et al. 1989).

Approximately 0.5 μg of total DNA from each individual was digested with the following 14 enzymes: *Apa* I, *Ava* I, *Bgl* I, *Dra* I, *Eco*R I, *Eco*R V, *Hin*d III, *Pst* I, *Pvu* II, *Sac* I, *Sca* I, *Sma* I, *Ssp* I, and *Xho* I. The resulting DNA fragments were electrophoretically separated in 0.8–1.2% agarose gels and transferred to a nylon membrane (positively charged, Boehringer Mannhaim Biochemicals). mtDNA fragments were visualized on the membrane by Southern blot analysis (Southern 1975) using a mixture of cloned and labeled inserts. Prehybridization and hybridization were at 68°C and final wash stringencies were 0.1 SSC – 0.1% SDS at 65°C for 20 min.

The restriction fragments were assigned molecular weights by comparing their mobilities with those of a size standard (a mixture of *Eco*R I and *Hin*d III digested lambda phage DNA). We used two methods for restriction site mapping: the double-digest technique (Sambrook et al. 1989) and selective probing of Southern blots with each single-cloned mtDNA *Hin*d III fragment.

Phylogenetic analyses

Composite binary haplotypes (presence or absence of restriction sites) were generated from the inferred restriction site data for each enzyme using the GENERATE program in the REAP computer package (McElroy et al. 1992). Nucleotide divergences among haplotypes were estimated based on the formulae of Nei and Tajima (1983) in the D program of REAP. A neighbor-joining (NJ) tree (Saitou and Nei 1987) was generated using the matrix of the nucleotide divergence between haplotypes with the NEIGHBOR program of PHYLIP, version 3.5c (Felsenstein 1993).

Composite binary haplotype data were also used in character-state approaches. The data was bootstrapped (Felsenstein 1985) using the PHYLIP SEQBOOTS program. A majority-rule consensus of the Wagner parsimony networks was constructed with the 1000 bootstrap samples using the MIX, DOLLOP, and CONSENSE modules of PHYLIP. We also carried out a maximum likelihood procedure in conjunction with the Kishino–Hasegawa test (Kishino and Hasegawa 1989) applying the PHYLIP RESTML program to verify the evolutionary hypotheses derived from the NJ tree and the majority-rule consensus. The tree topology based on the majority-rule consensus was compared with the tree topology constructed from the alternative evolutionary hypotheses by the Kishino–Hasegawa test.

Intrapopulation diversity

Individuals were added to several populations to increase sample sizes for estimation purposes; only variable restriction sites were screened. The mapped restriction site data in each of the following seven enzymes were used for calculations: *Apa* I, *Ava* I, *Dra* I, *Eco*R V, *Hin*d III, *Sac* I, and *Ssp* I. The haplotype and nucleotide diversity values (Nei and Miller 1990) within populations were calculated using the DA program of REAP (McElroy et al. 1992). The choice of this reduced set of enzymes was based on the number of informative restriction sites observed in phylogenetic analysis. To estimate the history of population dynamics in each population, the distribution of pairwise restriction site differences was also examined.

Results

mtDNA phylogenies

No morphologically intermediate individual was found in the sympatric sites of *P. pungitius* and *P. tymensis*. Every sample was unambiguously identified as either typical *P. pungitius* or typical *P. tymensis* based on their morphological characteristics. Two to 13 cleavage patterns were detected with each enzyme (Appendix A), yielding a total of 115 restriction sites. Thirty-seven mtDNA haplotypes were recognized from composite cleavage patterns (Table 1). Binary haplotypes based on the presence or absence of restriction sites were generated (Appendix B). Twenty-four of 37 haplotypes were found only in *P. pungitius* and the other 10 haplotypes were found in *P. tymensis*. The three remaining haplotypes (Nos. 10, 11, and 25) were observed in both species.

The NJ tree based on nucleotide divergences between the haplotypes revealed two major clusters (groups A and B)

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Table 1. Population and sample size of 37 *Pungitius* mtDNA haplotypes.

Haplotype	Fragment pattern														Population		
															River	Species	*N*
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Kunebetsu	*pungitius*	4
2	A	A	D	A	A	A	A	A	A	A	A	A	A	A	Kunebetsu	*pungitius*	2
3	B	C	A	A	A	C	B	A	A	A	A	A	J	A	Teshio	*pungitius*	4
															Mobetsu	*pungitius*	3
4	B	C	A	A	A	B	D	A	A	A	A	A	J	A	Teshio	*pungitius*	5
5	B	C	A	A	A	B	B	A	A	A	A	A	J	A	Teshio	*pungitius*	1
6	B	D	A	B	A	C	B	A	A	A	A	A	J	A	Teshio	*tymensis*	2
7	B	A	A	A	B	E	B	A	A	A	A	A	C	A	Teshio	*tymensis*	3
8	C	A	A	A	B	A	B	B	A	B	A	A	J	A	Teshio	*tymensis*	3
9	C	A	A	A	B	D	B	B	A	A	A	A	C	A	Teshio	*tymensis*	2
10	B	A	A	E	A	F	B	A	A	A	B	F	J	A	Osatsu	*pungitius*	9
															Osatsu	*tymensis*	8
															Uryu	*tymensis*	3
11	B	B	A	E	A	F	B	A	A	A	B	F	J	B	Osatsu	*pungitius*	3
															Osatsu	*tymensis*	3
12	B	A	A	E	A	G	B	A	A	A	B	F	J	A	Osatsu	*tymensis*	1
13	B	A	B	A	B	C	B	A	A	A	A	A	J	A	Biwase	*pungitius*	4
															Bekanbeushi	*pungitius*	1
14	B	A	C	F	B	H	B	A	A	A	A	A	B	C	Biwase	*pungitius*	2
															Bekanbeushi	*pungitius*	3
15	B	A	B	A	B	B	B	A	A	A	A	A	J	A	Biwase	*pungitius*	4
															Bekanbeushi	*pungitius*	2
16	G	E	D	A	A	J	B	B	A	C	B	B	E	A	Lyutoga	*pungitius*	3
															Bakhura	*pungitius*	3
17	C	A	D	A	A	A	B	A	A	A	A	A	A	A	Momokawa	*pungitius*	3
18	B	A	D	A	A	A	B	A	A	A	A	A	A	A	Arayadaira	*pungitius*	3
															Nakajo	*pungitius*	2
19	B	M	D	A	A	Q	B	A	A	A	A	A	A	A	Shokawa	*pungitius*	3
20	B	A	D	A	A	C	B	A	A	A	A	A	H	A	Ola	*pungitius*	1
21	B	A	D	A	A	H	B	A	A	A	A	A	H	A	Ola	*pungitius*	2
22	C	A	D	A	A	H	B	A	A	A	A	A	G	A	Recheka	*pungitius*	1
23	G	E	D	A	A	J	B	B	A	C	B	B	F	A	Recheka	*pungitius*	1
24	G	E	E	A	A	J	B	B	A	C	B	B	F	A	Recheka	*pungitius*	1
25	B	C	D	A	B	C	B	A	A	A	A	A	J	A	Tonbetsu	*tymensis*	6
															Yuchi	*pungitius*	2
															Koetoi	*pungitius*	2
															Horobetsu	*pungitius*	1
26	D	A	D	A	A	A	B	A	A	A	A	A	I	A	Nakajo	*pungitius*	1
27	B	A	D	A	A	D	B	A	A	A	A	A	C	A	Yuchi	*tymensis*	6
28	H	C	D	A	B	C	B	A	A	A	A	A	B	A	Yuchi	*pungitius*	1
29	B	C	D	A	B	C	B	A	A	A	H	A	J	A	Tonbetsu	*pungitius*	1
30	B	C	D	I	B	C	B	A	A	A	A	A	J	A	Tonbetsu	*pungitius*	2
31	B	A	E	A	A	C	B	E	A	A	A	A	J	A	Koetoi	*pungitius*	1
															Horobetsu	*pungitius*	2
32	B	A	D	A	A	C	B	A	A	C	A	A	J	A	Bolishaya	*pungitius*	1
33	B	A	D	A	A	H	B	A	A	C	A	A	J	A	Mikija	*pungitius*	1
34	X	X	X	X	X	X	X	X	X	Z	X	X	X	X	Biwase	*tymensis*	3
35	X	X	X	X	X	X	X	X	X	Z	X	X	Y	X	Biwase	*tymensis*	7
															Bekanbeushi	*tymensis*	6
															Bettoga	*tymensis*	2
36	Y	X	X	X	X	X	X	X	X	Z	X	X	Y	X	Bettoga	*tymensis*	1
37	X	Y	X	X	X	X	X	X	X	X	X	X	Y	Y	Nayba	*tymensis*	3
															Lyutoga	*tymensis*	3
															Koetoi	*tymensis*	3

Note: Letters indicate fragment patterns produced by the following enzymes: *Apa* I, *Ava* I, *Bgl* I, *Dra* I, *Eco*R I, *Eco*R V, *Hin*d III, *Pst* I, *Pvu* II, *Sac* I, *Sca* I, *Sma* I, *Ssp* I, and *Xho* I.

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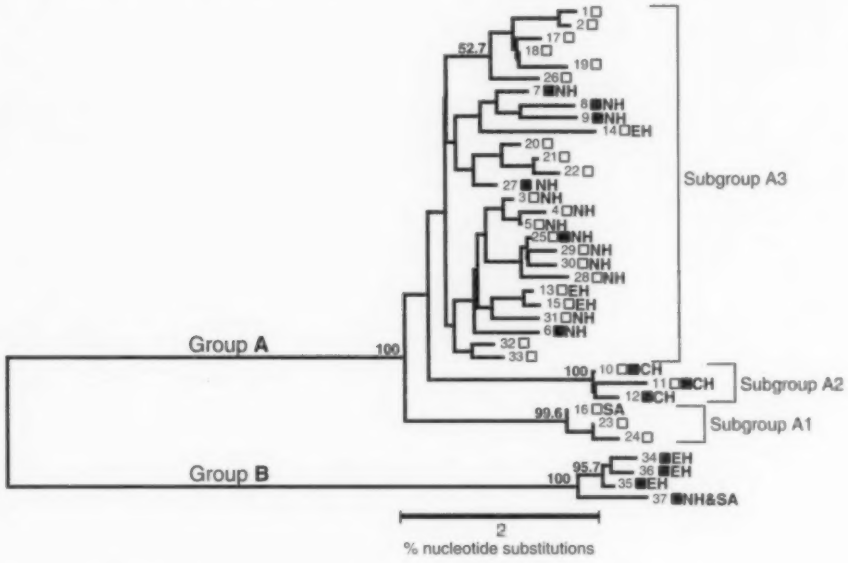
Fig. 2. NJ tree based on nucleotide divergences among 37 mtDNA haplotypes of *P. pungitius* and *P. tymensis*. The open and solid squares indicate mtDNA haplotypes carried by morphological *P. pungitius* and *P. tymensis*, respectively. The tree shows the divergences and relationships among the three subgroups within group A described in the text. The numbers on the branches indicate the bootstrap percentages (>50) that support each node of the tree, and the numbers at the tips of the branches indicate the haplotype numbers as given in Table 1.



separated by a mean nucleotide divergence of 5.94% (Fig. 2). All 24 haplotypes specific to *P. pungitius* clustered into group A, whereas four of 10 haplotypes specific to *P. tymensis* (Nos. 34, 35, 36, and 37) in the Lyutoga, Nayba, Koetoi, Bettoga, Biwase, and Bekanbeushi rivers formed group B, and the other six haplotypes (Nos. 6, 7, 8, 9, 12, and 27) in the Teshio, Osatsu, and Yuchi rivers and all three haplotypes common to both species (Nos. 10, 11, and 25) belonged in group A. These clustering patterns showed that the mtDNA lineages did not correspond completely to morphological species because the mtDNA haplotypes of *P. tymensis* were split between groups A and B.

Intragroup phylogeny in group A

Within group A, mtDNA haplotypes formed three subclusters (Fig. 2). The constituent haplotypes in the three subclusters were haplotypes of *P. pungitius* in Sakhalin and two of three individuals in the Recheka River (Nos. 16, 23, and 24) (subgroup A1), haplotypes specific to *P. tymensis* (No. 12) and common to the both species (Nos. 10 and 11) in the Uryu and Osatsu rivers (subgroup A2), and the rest of the haplotypes (subgroup A3).

To inspect the subgroupings, we reconstructed the phylogenetic relationships among the 37 mtDNA haplotypes using maximum-parsimony (MP) methods and bootstrapping (1000 replications). The resultant topology of the majority-rule consensus tree was similar to the NJ tree and showed that subgroups A1, A2, and A3 were monophyletic units (tree length 110 steps). High bootstrap probabilities (100%) supported subgroups A1 and A2, but subgroup A3 was supported by the low bootstrap probability (<30%) (Fig. 2). Haplotypes specific to *P. tymensis* and common to both species could be separated into at least two monophyletic lineages within group A.

Polyphyleticism was statistically tested by comparing trees using a maximum likelihood estimate in conjunction with the Kishino–Hasegawa test. Comparison was made between the topologies of the MP tree and the shortest tree among same haplotypes showing that all haplotypes specific to *P. tymensis* and common to both species in group A were monophyletic (tree length 118 steps). The result of the tree comparisons showed that the MP tree was the best and the difference in the log likelihood ratio between the MP tree and the hypothetical tree was –25.81 with a standard error of 10.25. Thus, the alternative phylogenetic hypotheses (monophyly of mtDNA haplotypes of *P. tymensis* in group A) were rejected by the Kishino–Hasegawa test.

Intrapopulation diversity

To examine the effect of historic population sizes from the present-day genetic composition of the populations, which carried group A and B mtDNA haplotypes in *P. tymensis*, intrapopulation diversities of the mtDNA were computed for the Lyutoga, Teshio, Koetoi, Biwase, Bekanbeushi, Uryu, and Osatsu River populations. The populations were chosen using three criteria related to the phylogenetic status of their mtDNA haplotypes. First, they belonged in group B (the Lyutoga, Koetoi, Biwase, and Bekanbeushi rivers). Second, they branched from a single node in group A (Uryu and Osatsu rivers). Third, they branched from the multiple nodes in group A (Teshio River). Then, the sympatric *P. pungitius* populations were appended except for two populations for which the sample sizes were insufficient (Koetoi and Bekanbeushi rivers).

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Table 2. Haplotype (h) and nucleotide (π) diversity (mean ± SE) within populations of *P. tymensis* and *P. pungitius*.

Population	Haplotype							N	h	π
P. tymensis										
Lyutoga	X	Y	X	X	X	Y	Y	15		
	X	Y	X	X	X	X	Y	9		
	X	Z	X	X	X	X	Y	8		
	X	Y	X	X	X	Z	Y	3		
							Total	34	0.6970±0.0433	0.001770
Koetoi	X	Z	X	X	X	X	Y	30		
							Total	30	0.0000±0.0000	0.000000
Teshio	C	A	A	A	B	B	J	14		
	C	A	A	D	B	A	C	10		
	B	A	A	E	B	A	J	10		
	B	A	A	E	B	A	C	6		
	L	C	A	B	B	A	J	5		
	B	C	D	C	B	A	J	5		
	C	A	A	A	B	B	C	3		
	B	D	B	C	B	A	J	3		
	B	A	D	D	B	A	C	2		
	K	A	A	A	B	B	J	2		
	C	A	A	D	B	A	J	1		
	N	A	A	A	B	B	J	1		
	C	A	A	A	B	B	L	1		
	C	P	A	A	B	B	B	1		
							Total	64	0.8889±0.0183	0.008112
Biwase	X	X	X	X	X	Z	X	17		
	X	X	X	X	X	Z	Y	7		
	Y	X	X	X	X	Z	X	2		
	X	X	X	X	X	Z	Z	1		
							Total	27	0.5499±0.0853	0.001123
Bekanbeushi	X	X	X	X	X	Z	X	20		
Total								20	0.0000±0.0000	0.000000
Uryu	B	A	G	F	B	A	J	28		
	B	A	E	F	B	A	J	2		
							Total	30	0.1287±0.0792	0.000248
Osatsu	B	A	E	F	B	A	J	16		
	A	B	E	F	B	A	J	8		
	B	A	E	G	B	A	J	4		
	B	B	E	F	B	A	J	2		
							Total	30	0.6437±0.0670	0.002123
P. pungitius										
Lyutoga	G	E	A	J	B	C	E	27		
	G	E	A	J	H	C	E	6		
	G	S	A	J	B	C	E	1		
							Total	34	0.3476±0.0898	0.000783
Teshio	B	C	A	C	B	A	J	13		
	B	C	A	B	D	A	J	11		
	B	C	A	B	B	A	J	1		
							Total	25	0.5567±0.0471	0.002129
Biwase	B	A	A	C	B	A	J	17		
	B	A	A	B	B	A	J	1		
	G	A	F	H	B	A	B	3		
	B	A	F	H	B	A	B	1		
	G	R	I	H	G	A	B	1		
							Total	28	0.7938±0.0391	0.004608
Osatsu	B	A	E	F	B	A	J	28		
	B	A	E	G	B	A	J	1		
	B	Q	E	F	B	A	J	1		
							Total	30	0.1310±0.0821	0.000263

Note: Letters indicate the fragment patterns produced by the following enzymes: *Apa* I, *Ava* I, *Dra* I, *Eco*R V, *Hind* III, *Sac* I, and *Ssp* I.

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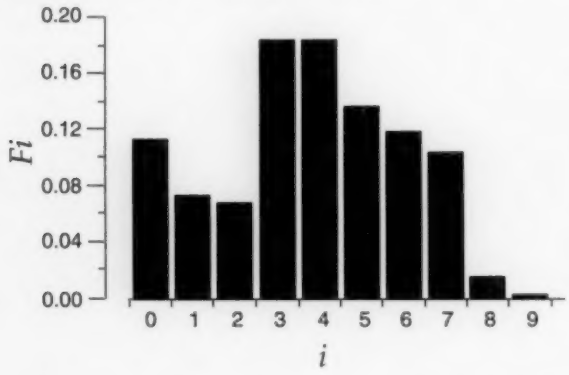
Fig. 3. Frequency distribution of pairwise restriction site differences observed in the *P. tymensis* population of the Teshio River. *Fi* denotes the probability that two random pair of mtDNA haplotypes differ by *i* restriction site changes.



The genetic composition of each population and the haplotype and nucleotide diversity values within populations are presented in Table 2. These rates varied among populations and ranged between 0.0000 and 0.8889 in haplotype diversity and from 0.00 000 to 0.00 811 in nucleotide diversity. The highest rate was recorded in the *P. tymensis* population of the Teshio River, which had group A mtDNA haplotypes. In contrast, there was no mtDNA variation within *P. tymensis* populations of the Koetoi and Bekanbeushi rivers, which carried group B mtDNA halotypes. The values of haplotype diversity in sympatric *P. tymensis* and *P. pungitius* populations in the Teshio River were 0.8889 and 0.5567, respectively. Haplotypic diversity was significantly higher ($t = 4.082$, $p < 0.001$) in the *P. tymensis* populations than in the *P. pungitius* populations. A comparison of sympatric *P. tymensis* and *P. pungitius* in the Osatsu River, the values of haplotype diversity within each population being 0.6437 and 0.1310, respectively, showed a higher significance ($t = 4.838$, $p < 0.001$) in *P. tymensis* than in *P. pungitius*. To estimate the history of population dynamics in the populations, the distribution of pairwise restriction site differences was also examined. In the *P. tymensis* population of the Teshio River, the histogram exhibited a wave with a crest at three site differences (Fig. 3), which implicated the past exponential growth (Rogers and Harpending 1992). In the other populations, however, the same waves were not detected in the distribution of pairwise restriction site differences (data not shown).

Discussion

Although occasional natural hybridization between *P. pungitius* and *P. tymensis* was detected in some rivers (Kobayashi 1957, 1959; Niwa 1987), the two species are generally reproductively isolated (Takata et al. 1984; Niwa 1987; Takata 1987). In this study, the mtDNA lineages were inconsistent with the species lineages, although phylogenetic analyses of their mtDNA represented two strikingly divergent lineages, namely groups A and B. The incongruity is that *P. tymensis* haplotypes were of both group A and B types, whereas all *P. pungitius* haplotypes were of group A type. Since group A was carried by all allopatric *P. pungitius* populations, including those by regions distant from the sympatric ranges, it is probably the authentic *P. pungitius* mitochondrial lineage. The question consequently arises as to why group A mtDNA was found not only in all *P. pungitius* populations but also in several *P. tymensis* populations.

Neigel and Avise (1986) concluded that the phylogenetic status for sister taxa when the maximum distance within each taxon is smaller than the minimum distance between the taxa is monophyly. For ninespine sticklebacks, divergence within both mtDNA haplotype groups was quite low compared with the difference between groups. This comparison indicates that the two mtDNA lineages have been embedded in the two isolated species for a sufficiently long time that postspeciation lineage sorting occurred. The prospect for retention of ancestral states and (or) convergent evolution of the mtDNA is inconceivable given the huge divergence. Horizontal transfer of mtDNA from *P. pungitius* is the other possible explanation for such gene tree – species tree discordances. Hybrids and back-cross progeny between *P. pungitius* and *P. tymensis* occasionally occur in nature (Kobayashi 1957, 1959; Niwa 1987), and laboratory F1 hybrids of this species pair were reciprocally fertile (Kobayashi 1959). The percentage of natural interspecies hybrids is about 3% (Niwa 1987), a rate sufficient to allow mtDNA gene flow between the species when used as the theoretical value for analysis according to Takahata and Slatkin (1984). Consequently, mtDNA introgression is the most likely explanation for the occurrence of mtDNA haplotypes of *P. tymensis* in group A.

Multiple lineages of the introgressed mtDNA

The gene tree among mtDNA haplotypes revealed that haplotypes of introgressed *P. tymensis* branched from multiple nodes within group A and the polyphyly was substantiated by the Kishino–Hasegawa test. All introgressed haplotypes from central Hokkaido were clustered with sympatric *P. pungitius* haplotypes and this monophyly was supported by a high bootstrap value (100%). In this region, a single population of *P. tymensis* collected from the Uryu River carried introgressed mtDNA haplotypes that had a very close relationship with the haplotypes in both species of the Osatsu River. The Uryu and Osatsu rivers are both tributaries of the Ishikari River, and continuous distribution of *P. tymensis* in this river system has been reported (Takata et al. 1984). The introgressed mtDNA haplotype in the two *P. tymensis* populations of central Hokkaido, therefore, could be derived from ancient introgression following dispersal of *P. pungitius* mtDNA within the *P. tymensis* populations in the Ishikari River system. In the Osatsu River, two mtDNA haplotypes that were not found in sympatric *P. pungitius* were found in the introgressed *P. tymensis* population. One possibility is to assume that the introgression occurred in the distant past and two haplotypes evolved within *P. tymensis*. Another possibility is the recent loss of the shared mtDNA haplotypes from the sympatric *P. pungitius* through a bottleneck. Nonetheless, it seems preposterous that the recent bottleneck affected only *P. pungitius* because *P. pungitius* were distributed more widely and continuously on Hokkaido than *P. tymensis* at the present time (Takata et al. 1984).

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On the other hand, the other introgressed mtDNA haplotypes found in the northern Hokkaido populations were scattered on the other clade. In this region, three of four river populations of *P. tymensis* (the Teshio, Yuchi, and Tonbetsu rivers) were introgressed and the remaining Koetoi River population had no introgression. Introgressant mtDNA haplotypes in the Yuchi and Tonbetsu River populations (Nos. 25 and 27) were identical to the sympatric and (or) neighboring allopatric *P. pungitius* haplotypes. Accordingly, the mtDNA introgression in these populations probably occurred relatively recently and originated from hybridization between sympatric *P. pungitius* and *P. tymensis*. On the other hand, no haplotypes common to the haplotypes of *P. pungitius* populations were found in the Teshio River. Branching orders of these haplotypes were polyphyletic in the NJ and MP trees, and genetic divergences between *P. pungitius* mtDNA haplotypes were larger than that between haplotype Nos. 25 and 27 of the Yuchi and Tonbetsu River populations. Thus, the origins of these introgressant haplotypes remain uncertain, but this may reflect an earlier origin for these haplotypes than for the other introgressed haplotypes common to *P. pungitius*. Judging from the above, the mtDNA introgression from *P. pungitius* is suggested to have occurred in the central Hokkaido region independently from that in the northern Hokkaido region.

Causes of the mtDNA introgression

Interestingly, the regional incidences of the *P. pungitius* mtDNA introgression differed distinctly among the four sympatric regions: although the introgression occurred in five of the six *P. tymensis* populations via at least three independent lines in northern and central Hokkaido, no introgression was found in the other regions. Such heterogeneity could be explained by the geographically variable patterns of selection against the introgressed mtDNA and (or) the genetic differences among the populations inhabiting each region that accumulated during historical isolation. Dowling et al. (1997) examined the regional differences in the direction of mtDNA introgression between the North American shiners *Luxilus cornutus* and *Luxilus chrysocephalus* and concluded that the genetic difference between the two regions (Lake Erie and Lake Michigan drainages) is responsible for the biased mtDNA introgression. It must also be noted that they suggested that the two regions do not differ with respect to any obvious physical or ecological parameters.

Previous allozymic studies of the ninespine sticklebacks revealed the absence of population subdivision within both *P. pungitius* and *P. tymensis* in Hokkaido (Niwa 1987; Takata 1987). In addition, the nucleotide divergences among the mtDNA haplotypes of the nonintrogressed *P. tymensis* in the three sympatric regions were less than 0.005 in the present study. These rates are quite small, as compared with the intraspecific mtDNA divergences observed in the two shiner species and *Culaea inconstans* between the Lake Erie and Lake Michigan drainages (Gach 1996; Dowling et al. 1997). On the other hand, the distribution of the mtDNA haplotypes of the three subgroups suggested that *P. pungitius* populations were geographically subdivided. This is not the case in *P. tymensis* and the differential dispersal could be potentially affecting the hybridization dynamics. However, the genetic differences in *P. pungitius* populations between the regions may not have affected the differences in the regional incidence of mtDNA introgression, at least for northern and eastern Hokkaido, because there are no genetic differences between these regions in the present study.

On the other hand, the possibility of geographically variable patterns of selection is difficult to rule out, since climatic factors vary among the regions. In particular, the monthly mean air temperatures during the breeding season of these species (April–July 1961–1990 base; Sapporo District Meteorological Observatory 1991) of central and northern Hokkaido are over 1°C higher than those of eastern Hokkaido and Sakhalin. Current and past climate warming inevitably reduces the spawning area for cold-adapted fish species such as the ninespine sticklebacks, particularly in the warm parts of their native ranges (Meisner 1990). Since both northern and central Hokkaido are "thermal" southern margins of the *P. tymensis* ranges, detrimental effects of such warming in these regions would be greater than those in the other regions. On the other hand, considering the latitudinally extensive ranges of *P. pungitius*, the effects of climatic warming on this species are probably less than those on *P. tymensis* across the overall sympatric regions.

The occurrence of natural hybridization is thought to be related to a disparity in the size of the parental populations (Hubbs 1955). The high incidences of the introgressive hybridization of *P. pungitius* mtDNA in the northern and central Hokkaido *P. tymensis* populations, therefore, may be attributable to the warm climate, which decreased especially *P. tymensis* abundance. Furthermore, in the absence of mate preference in the back-cross progenies, the direction of mtDNA introgression will tend toward the rare taxon from the more numerous taxon (Takahata 1986). The one-way mtDNA introgression, from *P. pungitius* to *P. tymensis*, would likely be caused by the difference in effective population sizes between the two species. In spite of the present abundances of *P. tymensis* in northern Hokkaido (Takata et al. 1984), the lack of intrapopulation mtDNA diversities observed only in the *P. tymensis* populations in the Koetoi River suggests that these populations have experienced a severe bottleneck or a founder effect in the past.

Outcomes

In contrast with the pure *P. tymensis* populations, mtDNA variability within the introgressed *P. tymensis* populations in the Teshio and Osatsu rivers was significantly higher than that observed in the sympatric *P. pungitius* populations. Furthermore, a wave in the distribution of pairwise restriction site differences and an excess of rare genotypes in *P. tymensis* of the Teshio River indicated the sudden population growth following the mtDNA introgression. Although mtDNA variability within populations could be enlarged by immigration of mtDNA variants (Takahata and Palumbi 1985), these are probably not as effective in the present mtDNA variation because no haplotypes common to the other populations were observed in this study. Furthermore, recall our suggestion that the mtDNA haplotypes observed in the Teshio River population may have originated from an old introgression event. This implies no present-day gene flow into the *P. tymensis* population in this river; and the mtDNA diversity is ascribed to selectional relaxation for the

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introgressed mtDNA genotypes. Fixation of the introgressed genotypes in a recipient population will be strongly accelerated by the reduction in effective population size (Maynard Smith 1989). Viewed in this light, the introgressed populations of *P. tymensis* have probably experienced a bottleneck similar to those of the pure populations of this species in northern Hokkaido.

Accordingly, the past introgressive hybridization perhaps caused the increase in mtDNA variety of introgressed populations, and these results support our prediction that introgressive hybridization occasionally increases the genetic variability in recipient populations at least at the mtDNA level. Arnold (1997) summarized the two contrasting viewpoints from consideration of natural hybridization as it relates to issues of conservation biology. The first regards natural hybridization as deleterious for biodiversity, and thus the cause of loss of rare taxa. The second is that hybridization between rarer and more numerous taxa potentially results in a genetic enrichment of the endangered form. Our results, that the mtDNA varieties have been restored only in the introgressed populations but not in the pure populations, provide strong support for the second viewpoint: the recent introgressive hybridization may have salvaged the genetic diversity of *P. tymensis* populations in the southern margins of their native ranges.

In fish, natural introgressive hybridization is relatively widespread, but only a few attempts have been made to determine the evolutionary significance of this phenomenon (Dowling and DeMarais 1993; Dowling et al. 1997). The present findings provide new information on the evolutionary impacts of introgressive hybridization in the genetic structure of recipient populations. Furthermore, our results should propose an instructive research direction that the comparison of the genetic diversity among the multiple introgressant lineages may contribute to clarifying the evolutionary importance of introgressive hybridization among fish species. The further accumulation of data on hybridizing fish species would help us to understand the general significance of natural hybridization and introgression.

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Appendix A. Size of mtDNA restriction fragments produced by 14 enzymes used in the present study.

Apa I	A	B	C	D	G	H	K	L	N	X	Y
						7.78					
							7.50				
			6.20						6.20		6.20
										5.20	5.20
	4.65	4.65		4.65	4.65	4.65		4.65			
	3.90	3.90	3.90				3.90	3.90	3.90		
		3.88	3.88	3.88			3.88	3.88			
					3.75						
				3.15	3.15					3.15	
										3.05	
	2.78								2.78		
				2.32				2.32			
	1.55	1.55		1.55	1.55	1.55		1.55			
	1.30	1.30	1.30		1.30	1.30			1.30	1.30	1.30
	1.10								1.10		
	1.02	1.02	1.02		1.02	1.02	1.02		1.02	1.02	1.02
										1.00	1.00
										0.83	0.83
				0.75	0.75					0.75	0.75
					0.13						
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30

Ava I	A	B	C	D	E	M	P	Q	R	S	X	Y	Z
	3.55	3.55	3.55	3.55	3.55	3.55		3.55	3.55				
							3.25			3.25			
	3.20	3.20	3.20	3.20	3.20		3.20	3.20	3.20	3.20			
	2.70	2.70	2.70			2.70				2.70			
											2.60	2.60	2.60
												2.55	2.55
								2.35					
	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
	2.15		2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15			
	2.10	2.10		2.10	2.10	2.10	2.10		2.10	2.10	2.10	2.10	2.10

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Appendix A (*continued*).

Ava I	A	B	C	D	E	M	P	Q	R	S	X	Y	Z
									2.10		2.10	2.10	2.10
						1.75							
			1.60					1.60					
				1.55									
						1.45							
											1.45	1.45	1.45
											1.40		
					1.38		1.38						
											1.35	1.35	1.35
					1.32		1.32						
				1.15									
											1.15		
		1.10											
		1.05											
											0.95	0.95	
									0.60		0.60	0.60	0.60
													0.58
			0.50					0.50					
													0.37
								0.35					
							0.30			0.30			
	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30

Bgl I	A	B	C	D	E	X
						8.33
				7.10	7.10	
		6.85			6.85	
	5.90		5.90	5.90		5.90
	4.95	4.95	4.95			
			2.35			
	2.15	2.15	2.15			
	1.23	1.23		1.23	1.23	
	1.12	1.12		1.12	1.12	1.12
	0.95		0.95	0.95		0.95
Total	16.30	16.30	16.30	16.30	16.30	16.30

Dra I	A	B	D	E	F	G	I	X
							8.70	
	6.15	6.15	6.15	6.15	6.15	6.15		
								5.40
		4.70	4.70					4.70
	3.95						3.95	
				2.75				
	2.55	2.55	2.55	2.55	2.55	2.55		2.55
					2.05			
						2.02		
				1.95		1.95		
					1.90			
	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
	1.35		1.35	1.35	1.35	1.35	1.35	1.35
		1.15						
	0.75				0.75		0.75	0.75
						0.73		
		0.20						
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30

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Appendix A (*continued*).

*Eco*R I	A	B	X
		16.30	
	8.55		
	7.75		7.75
			7.75
			0.80
Total	16.30	16.30	16.30

*Eco*R V	A	B	C	D	E	F	G	H	J	Q	X
				10.85							
			8.95					8.95			
											7.10
											6.95
		6.35	6.35						6.35		
	5.95	5.95			5.95	5.95	5.95		5.95	5.95	
					4.90						
	4.45			4.45	4.45	4.45	4.45	4.45		4.45	
	3.00	3.00				3.00					
							2.10				
										2.00	
	1.90							1.90		1.90	
									1.85		
											1.70
						1.40	1.40				
									1.15		
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
										1.00	
							0.90				
											0.55
						0.50	0.50				
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30

Hind III	A	B	D	G	H	X
						6.45
			5.00			
	3.60	3.60		3.60	3.60	
	3.50	3.50	3.50	3.50	3.50	
	3.20		3.20			
		2.50		2.50		2.50
	2.35	2.35	2.35	2.35	2.35	2.35
						2.05
	1.70	1.70	1.70		1.70	1.70
					1.50	
	1.40	1.40		1.40	1.40	
				1.05		
					1.00	
		0.70		0.70	0.70	0.70
				0.65		
	0.55	0.55	0.55	0.55	0.55	0.55
Total	16.30	16.30	16.30	16.30	16.30	16.30

Pst I	A	B	E	X
				10.75
	9.23	9.23	9.23	
		7.07		
	5.55			5.55
			5.15	
	1.52		1.52	
			0.40	
Total	16.30	16.30	16.30	16.30

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Appendix A (*continued*).

Pvu II	A	X
		7.73
		5.21
	4.38	
	4.05	
	3.68	
	2.60	2.60
	0.83	
	0.76	0.76
Total	16.30	16.30

Sac I	A	B	C	X	Z
		10.10			
	8.15		8.15	8.15	8.15
	5.50	5.50			5.50
			2.76	2.76	
			2.74	2.74	
				2.65	2.65
	1.95		1.95		
	0.70	0.70	0.70		
Total	16.30	16.30	16.30	16.30	16.30

Sca I	A	B	H	X
		7.59		
				5.76
	4.98		4.98	4.98
			4.51	
	4.20	4.20	4.20	
	3.92	3.92		
				2.90
	2.61		2.61	
				1.39
				1.27
	0.59	0.59		
Total	16.30	16.30	16.30	16.30

Sma I	A	B	C	D	E	F	X
	16.30						
				12.00			
						10.70	
			9.40				
		9.12					
					8.45		8.45
		7.18					
			6.90				
						5.60	
							3.70
					3.55		3.55
				2.25	2.25		
				1.45	1.45		
				0.60	0.60		0.60
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30

Ssp I	A	B	C	E	F	G	H	J	L	X	Y	Z
									7.25			
	5.40	5.40	5.40	5.40	5.40	5.40	5.40	5.40				
	4.60		4.60				4.60	4.60	4.60	4.60	4.60	4.60
	4.05	4.05	4.05	4.05		4.05	4.05	4.05	4.05	4.05		4.05
										3.55	3.55	
		3.05		3.05	3.05	3.05						

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Appendix A (*continued*).

Ssp I	A	B	C	E	F	G	H	J	L	X	Y	Z
					2.88							
											2.15	2.15
											1.90	1.90
		1.85		1.85	1.85			1.85		1.85	1.85	1.85
						1.78	1.78					
		1.55		1.55	1.55	1.55						
										1.35	1.35	1.35
					1.17							
	0.95		0.95									
	0.90		0.90									
										0.50	0.50	
			0.40	0.40	0.40	0.40	0.40					
	0.22	0.22						0.22	0.22	0.22	0.22	0.22
	0.18	0.18						0.18	0.18	0.18	0.18	0.18
						0.07	0.07					
Total	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30	16.30

Xho I	A	B	C	X	Y
					9.75
	8.85	8.85	8.85	8.85	
		7.45			
	5.35		5.35		
				3.75	3.75
	2.10			2.10	
				1.60	1.60
					1.20
			1.05		
			1.05		
Total	16.30	16.30	16.30	16.30	16.30

Note: Letters indicate fragment patterns produced by the following enzymes: *Apa* I, *Ava* I, *Bgl* I, *Dra* I, *Eco*R I, *Eco*R V, *Hind* III, *Pst* I, *Pvu* II, *Sac* I, *Sca* I, *Sma* I, *Ssp* I, and *Xho* I. Sizes are in kilobase pairs and corrected by restriction site mapping. Empty cells indicate fragments absent.

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Appendix B. Presence (1) – absence (0) matrix of restriction sites for 37 *Pungitius* mtDNA haplotypes.

		Haplotype No.																	
	Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Apa I	2.70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.00	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1
	8.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9.65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	10.95	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11.97	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	12.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	12.80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14.75	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15.85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ava I	0.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.60	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5.65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.90	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	7.45	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	8.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8.60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	10.85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11.95	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	13.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16.20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Bgl I	2.25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3.20	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
	9.10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	10.22	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
	11.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	13.60	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
Dra I	0.70	1	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1	1
	1.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3.70	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
	9.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	9.85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11.40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	13.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15.10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	15.80	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
*Eco*R I	7.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16.00	1	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1
*Eco*R V	4.85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10.30	1	1	0	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1

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Haplotype No.																		
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	0	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0

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Appendix B (*continued*).

		Haplotype No.																	
	Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	11.70	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
	12.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12.20	1	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	1
	14.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14.30	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	14.65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15.20	1	1	0	1	1	0	1	1	0	1	1	1	0	0	1	1	1	1
Hind III	0.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3.80	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.55	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	9.90	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13.65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15.20	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pst I	5.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6.90	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1
	12.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pvu II	1.75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8.03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8.79	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	13.17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	14.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sac I	3.10	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
	5.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8.51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	11.25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sca I	1.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.07	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3.68	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	1
	8.66	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	9.25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	13.17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	14.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sma I	0.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	10.70	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Ssp I	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5.95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	9.50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	11.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	11.27	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1
	11.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

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Haplotype No.																		
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

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Appendix B (*concluded*).

		Haplotype No.																	
	Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	12.40	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	13.23	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	13.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	15.50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Xho I	5.70	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11.05	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
	12.10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	12.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Note: Mapped positions of restriction sites are in kilobase pairs, with the conserved *Sma* I site arbitrarily chosen as the zero point.

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Haplotype No.																		
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

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Modeling relationships between landscape-level attributes and snorkel counts of chinook salmon and steelhead parr in Idaho

William L. Thompson and Danny C. Lee

Abstract: Knowledge of environmental factors impacting anadromous salmonids in their freshwater habitats, particularly at large spatial scales, may be important for restoring them to previously recorded levels in the northwestern United States. Consequently, we used existing data sets and an information-theoretic approach to model landscape-level attributes and snorkel count categories of spring–summer chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) parr within index areas in Idaho. Count categories of chinook salmon parr were negatively related to geometric mean road density and positively related to mean annual precipitation, whereas those for steelhead parr were negatively related to percent unconsolidated lithology. Our models predicted that chinook salmon parr would be in low count categories within subwatersheds with >1 km·km^{-2} geometric mean road densities and (or) <700 mm mean annual precipitation. Similarly, steelhead parr were predicted to be in low count categories in subwatersheds with >30% unconsolidated lithology. These results provide a starting point for fish biologists and managers attempting to map approximate status and quality of rearing habitats for chinook salmon and steelhead at large spatial scales.

Résumé : La connaissance des facteurs environnementaux influant sur les salmonidés anadromes dans leurs habitats dulcicoles, particulièrement aux grandes échelles spatiales, peut être importante pour le rétablissement des populations aux niveaux observés dans le passé dans le nord-ouest des États-Unis. Ainsi, nous avons utilisé des ensembles de données déjà existants et une approche basée sur la théorie de l'information pour relier des attributs du paysage avec l'abondance des tacons de saumon quinnat (*Oncorhynchus tshawytscha*) et de saumon arc-en-ciel (*Oncorhynchus mykiss*), dénombrés dans l'eau par plongée au tuba, dans des secteurs témoins de l'Idaho. Les catégories d'abondance des tacons de saumon quinnat étaient corrélées négativement avec la moyenne géométrique de la densité des routes et corrélées positivement avec les précipitations anuelles moyennes, tandis que celles des tacons de saumon arc-en-ciel étaient corrélées négativement avec le pourcentage de matière non consolidée. Nos modèles prévoient que les tacons de saumon quinnat seraient peu abondants dans les bassins secondaires où les moyennes géométriques de la densité des routes sont >1 km·km^{-2} et (ou) les précipitations annuelles moyennes sont <700 mm. De même, ils prévoient que les tacons de saumon arc-en-ciel seraient peu abondants dans les bassins secondaires où le pourcentage de matière non consolidée est >30%. Ces résultats peuvent servir de point de départ aux biologistes et aux gestionnaires responsables de la faune ichthyenne qui veulent établir des cartes représentant l'état et la qualité approximatifs des habitats où se développent le saumon quinnat et le saumon arc-en-ciel couvrant de grandes régions.

[Traduit par la Rédaction]

Introduction

Numbers of anadromous salmonids have greatly decreased from previously recorded levels for many stocks in the northwestern United States (Nehlsen et al. 1991). For instance, numbers of salmon and steelhead in the Columbia Basin have decreased sharply from an estimated 10–16 million adults to about 1.5–4.0 million adults during this century (Northwest Power Planning Council 1986). One factor thought to be influencing these declines is loss or degradation of freshwater spawning and rearing habitats (Nehlsen et al. 1991). Unfortunately, empirical data supporting this assertion at the landscape or basinwide scale are lacking in the published literature because fishery research has traditionally been conducted on smaller spatial scales (Schlosser 1991; but see Dunham and Rieman 1999; Torgersen et al. 1999). Thus, there is a need for empirically based models to investigate relationships between large-scale habitat and land management attributes and numbers of anadromous salmonids in their rearing environments (e.g., Bradford et al. 1997). These models could be used to predict status and quality of salmon spawning and rearing habitats across an area of interest as well as serve to highlight possible factors affecting population status and trends.

Availability of broadscale habitat and land management data generated by the recent interior Columbia Basin assessment (Quigley and Arbelbide 1997) and a 10-year data set of

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spring–summer chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) parr counts obtained from streams across Idaho (Hall-Griswold and Petrosky 1996) provided an opportunity to build large-scale predictive models based on empirical data. Consequently, we applied the latest information-theoretic modeling techniques (Buckland et al. 1997; Burnham and Anderson 1998) to investigate possible relationships between broadscale habitat and land management attributes and snorkel counts of spring–summer chinook salmon and steelhead parr within index streams in the Snake River drainage in Idaho. This geographical area is of particular importance because the indigenous stocks of spring–summer chinook salmon and steelhead have been listed as threatened under the Endangered Species Act (Federal Register 1997, 1998*a*). Due to various shortcomings with the snorkel count data, emphasis of this paper is as much on the approach to extract information from this broadscale but problematic data set as it is on interpretation of model results. We emphasize that the information-theoretic approach to model building, model selection, and model averaging applied in this paper is relevant to any study requiring a statistically based modeling approach.

Materials and methods

Snorkel count data set

The Idaho Department of Fish and Game (IDFG) and several cooperating agencies conducted snorkel counts of juvenile chinook salmon and steelhead (i.e., parr) in the Salmon River, Clearwater River, and lower Snake River drainages in Idaho during 1986–1995 (Hall-Griswold and Petrosky 1996) (Fig. 1). Abundance indices were obtained via snorkel counts by divers swimming approximately 100 m upstream within stream sections. One to five divers were used depending on stream size (Petrosky and Holubetz 1986). Stream sections were chosen based on a variety of criteria such as access, existence of previous counts, and perceived quality of rearing habitat (J. Hall-Griswold, IDFG, Stanley, Idaho, personal communication). Thus, selection of stream sections was nonrandom, but these sections represented a spectrum of habitats, stocks, and production types (i.e., wild (native) and natural (having a previous hatchery influence); Rich and Petrosky 1994). Although an attempt was made to survey the same sections over time, location and size (length and width) of snorkeled sections often varied among years mainly due to loss of previous section boundary markers, difficulties in relocating inadequately described sections, loss of access, and annual differences in stream flows. Further, not all sections were surveyed every year because of personnel, funding, and logistical constraints (J. Hall-Griswold, IDFG, Stanley, Idaho, personal communication). Finally, some stream sections were stocked with hatchery fish to better evaluate population responses of parr to mitigation measures (Petrosky and Holubetz 1986).

Subsetting the snorkel count data set

We only analyzed counts from stream sections where mitigation measures and stocking were not applied (see Rich and Petrosky 1994, their appendix B) because of confounding effects of those factors on the relationship between landscape-level variables and fish abundance. In addition, we limited our analyses to counts conducted when the water temperature exceeded 9°C because of the low detectability of fish below this temperature (Thurow 1994), which also would have had a confounding effect on the relationship between landscape attributes and snorkel counts.

Because snorkel counts were uncorrected for incomplete detectability of fish within sections and therefore contained an unknown amount of bias (e.g., Rodgers et al. (1992) reported that only 40% of fish were detected during their snorkel counts), we pooled them into two categories in an attempt to alleviate detrimental effects of this bias on interpretation of model results. Categories were defined based on fish density indices and habitat ratings used by IDFG to categorize quality of rearing habitat; these values were 0.12 parr·m^{-2} for chinook salmon and 0.06 parr·m^{-2} for steelhead (Hall-Griswold and Petrosky 1996). Counts were divided by estimated area of each snorkeled stream section to provide a common unit of comparison with the IDFG ratings. Snorkel counts per unit area at or below 0.12 parr·m^{-2} for chinook salmon or 0.06 parr·m^{-2} for steelhead were placed into category 1, whereas higher counts per unit area were placed into category 2. This approach may lessen effects of bias, for instance, when two stream sections have the same actual densities of chinook salmon parr (e.g., 0.06 parr·m^{-2}) but different detection rates of individuals (e.g., 40 and 80%). In this case, if observed counts were used, one section would be improperly modeled with twice the observed count than the other section, which could lead to spurious model results. Conversely, results of both counts would be placed into the same count category under the categorization approach described above. Note, however, that categorization still could lead to misclassification of stream sections (and spurious results), depending on the detectability of fish within a given section and how close the count per unit area was to the cutoff value used in the categorization.

We pooled seasonal runs for both species because sample sizes were inadequate to model these data separately. Although peaks in average counts per unit area differed slightly between runs for both species in two years during 1986–1990, their 90% confidence intervals broadly overlapped. We also included both wild and natural populations in our analyses. Further, we concentrated on counts from C channels for chinook salmon and B channels for steelhead because these were their optimal habitats (Hall-Griswold and Petrosky 1996) and therefore should have supported higher densities of parr. C channels occurred in low-gradient (<2% slope) terrain, whereas B channels were those in moderate-gradient (2–4% slope) terrain (Rosgen 1985, 1996). Finally, data from the year with the highest average counts per unit area for each species were used in our investigation of landscape linkages. We did this to maximize our ability to detect a difference between better and lower quality sites, where quality was defined in terms of fish counts per unit area during years of high fish numbers. Preliminary analyses suggested that average counts per unit area of fish were similar between better quality sites and lower quality sites during years of low counts, whereas these sites were much more distinct during years of high counts (J. Peterson, Rocky Mountain Research Station, Boise, Idaho, unpublished data). Difficulties associated with site identification, nonrandom site selection, incomplete time series of surveys, and counting bias precluded use of typical methods for modeling time series data.

Landscape habitat and anthropogenic data

Landscape-level data were compiled by Lee et al. (1997) and defined at the subwatershed scale, which is about 7800 ha on average within the Columbia Basin. These variables were categorized as either physiographic and geophysical or as anthropogenic (Table 1). One of the anthropogenic variables, management cluster, was categorical (i.e., each subwatershed was assigned the predominant category) and was generated by Lee et al. (1997) from results of a cluster analysis of variables representing land-type classification, management classification, ownership, percent grazed, and percent wilderness (for details, see Lee at al. 1997). We further pooled these results into four broad categories for simplicity (Ta-

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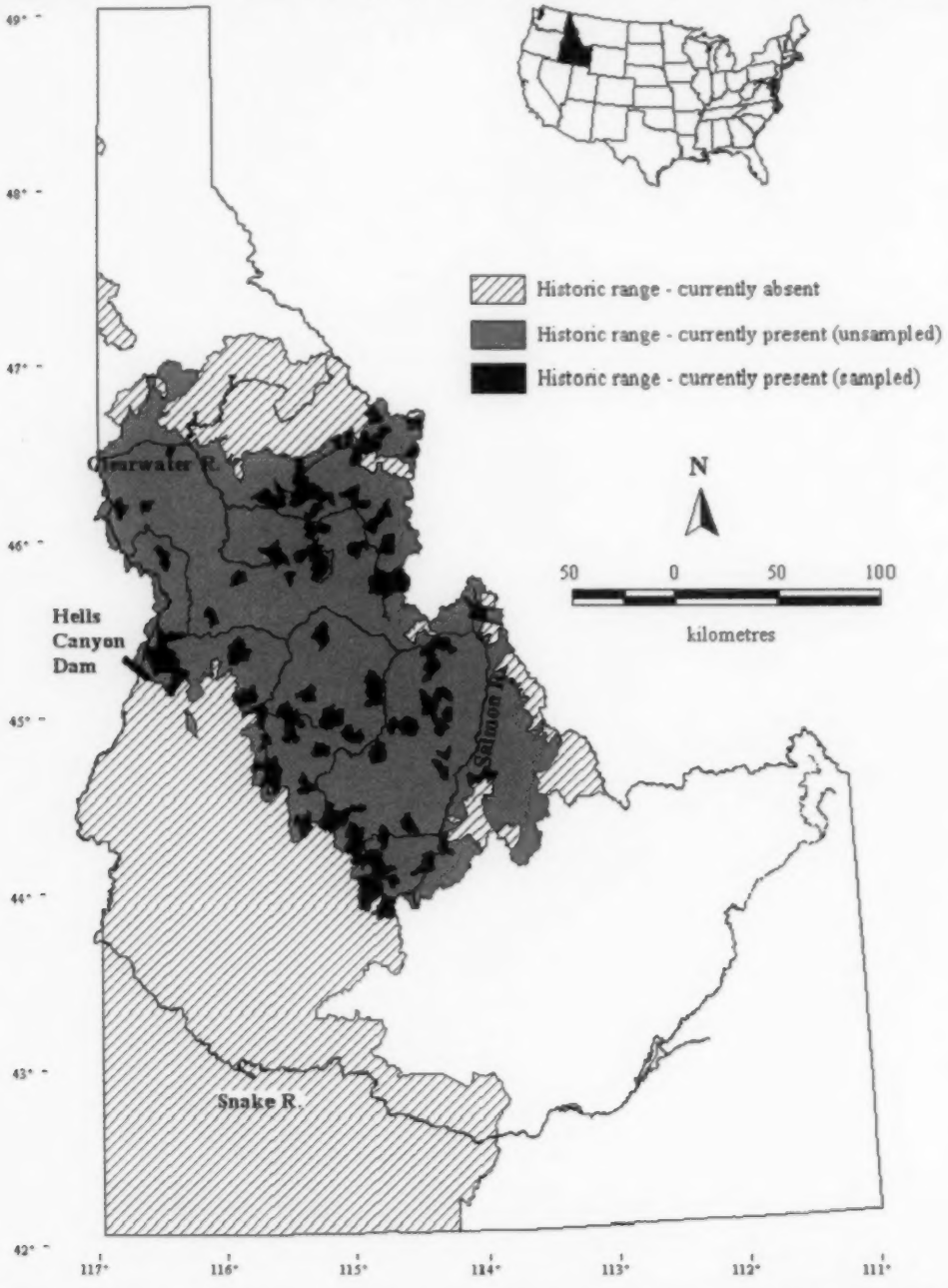
Fig. 1. Mapping of historical and current range of chinook salmon and steelhead in Idaho, U.S.A. (Lee et al. 1997). Dark areas are subwatersheds containing stream sections that were sampled for chinook salmon parr during 1987 and steelhead parr during 1990.



ble 1). This variable is an index to potential effects of land use and land management practices on adjacent streams and stream fish populations.

Modeling approach

We employed the information-theoretic approach to model building and selection suggested by Akaike (1973) and extended by Burnham and Anderson (1998). First, a global (i.e., overall; Burnham and Anderson 1998) logistic regression model was constructed with count category as the dichotomous response and landscape-level habitat and anthropogenic covariates that were deemed ecologically most relevant as predictors. Choice of predictors was based on results from Lee et al. (1997) and subject area experts familiar with the study area. We then assessed the fit of the global model via the Hosmer–Lemeshow goodness-of-fit (GOF) test and checked the Pearson χ^2 residuals for obvious outliers (i.e., >2; Hosmer and Lemeshow 1989). An outlier was dropped from

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Table 1. Category and description of covariates used in modeling landscape (subwatershed scale) habitat and land management attributes (Lee et al. 1997) with count categories of chinook salmon and steelhead parr.

Category	Model covariate	Description
Physiographic and geophysical	Precip	Mean annual precipitation (mm) based on the PRISM model (Daly et al. 1994)
	Sumtemp	Mean annual maximum summer temperature (°C)
	Slope	% of subwatershed with slopes >50%
	Mafic	% of subwatershed with mafic lithology
	Unconsol	% of subwatershed with unconsolidated lithology
Anthropogenic	Georoad	Geometric mean road density ($km \cdot km^{-2}$)
	Mngclus	Management cluster variable containing four land use and ownership categories: (1) HIF (high impact forest): high impact, grazed USDA Forest Service forest (2) MF (managed forest): moderate to high impact, ungrazed USDA Forest Service forest (3) W (wilderness): USDA Forest Service wilderness (4) R (rangeland): USDI BLM rangelands and moderate impact, grazed USDA Forest Service rangeland

analysis if its inclusion caused serious model lack of fit (see below). The Hosmer–Lemeshow GOF statistic was generated by ordering observations by their event probabilities, grouping them into a $2 \times g$ table (where g is number of groups; for the grouping procedure, see Hosmer and Lemeshow 1989), and calculating a Pearson χ^2 GOF statistic for this table. Low P values ($P < 0.10$) indicated model lack of fit. If the global model adequately fitted the data, we constructed a subset of candidate models from it that represented ecologically meaningful combinations of the landscape covariates. Each subsetted model was assumed to provide an adequate fit if the global model did so (Burnham and Anderson 1998).

Model selection was performed using a modification of Akaike's information criterion (AIC) (Akaike 1973; Burnham and Anderson 1998). An extension of likelihood theory, AIC is an estimate of the relative distance between model pairs (Burnham and Anderson 1998), where distance refers to the Kullback–Leibler distance of information theory (Kullback and Leibler 1951). The Kullback–Leibler distance is a measure of the degree of information loss when a model is used to approximate reality (Cover and Thomas 1991; Burnham and Anderson 1998). Specifically, AIC is defined as

$$\mathrm{AIC} = -2\ \ln(L(\hat{\theta}|\ \mathrm{data})) + 2k$$

where $\ln(L(\hat{\theta}|\ \mathrm{data}))$ is the maximized log-likelihood over the unknown model parameters (θ) given the data and k is the number of estimable parameters in the model (Buckland et al. 1997; Burnham and Anderson 1998). We used the small sample adjustment to the AIC that also corrects for overdispersion in count data, called QAICc. This statistic is calculated as

$$\mathrm{QAICc} = \frac{-2\ln(L(\hat{\theta}|\ \mathrm{data}))}{\hat{c}} + 2k + \frac{2k(k+1)}{n-k-1}$$

where $\hat{c}$ is the χ^2 GOF statistic for the global model and n is sample size (Burnham and Anderson 1998). Overdispersion refers to instances where sampling (observed) variance exceeds the theoretical variance of the underlying model (e.g., binomial model) and is commonly present in count data (Burnham and Anderson 1998). We used $\hat{c}$ to adjust for overdispersion in parameter estimates for each candidate model as well.

Models with lower QAICc values are considered better approximating models than those with higher values. However, QAICc is a relative statistic. The meaningful quantity for comparing candidate models is the difference between a particular model's QAICc value and the lowest QAICc value from all models; this difference is referred to as ΔQAICc (Burnham and Anderson 1998). The relative plausibility or weight of evidence of each model, given the data (w_i), can then be computed as

$$w_i = \frac{e^{(-\Delta\mathrm{QAICc}_i/2)}}{\sum_{r=1}^{R} e^{(-\Delta\mathrm{QAICc}_r/2)}}$$

where $\Delta\mathrm{QAICc}_i$ is the ΔQAICc value for the ith model in a set of R candidate models (Buckland et al. 1997). These w_i, or model weights, also can be used in model averaging. Instead of assuming a single "best" model and using its parameter estimates to make inferences, we based our inferences and predictions on a composite model generated from the w_i weighted average of parameter estimates for each landscape covariate from the set of candidate models (for details on model averaging, see Burnham and Anderson 1998). Model averaging incorporates both uncertainty related to model selection and uncertainty associated with parameter estimates within each candidate model. Inference based on a single model will lead to underestimates of variance and hence poor confidence interval coverage for parameter estimates unless its w_i is much higher (see below) than that of all other competing models (Burnham and Anderson 1998). Our composite models (one each for chinook salmon and steelhead data) only contained landscape covariates within candidate models whose w_i were at least one tenth of the maximum w_i, which is comparable with the minimum cutoff point (i.e., 8 or 1/8) suggested by Royall (1997) as a general rule-of-thumb for evaluating strength of evidence.

Interpreting model results

Data for landscape covariates were standardized so that their coefficients could be interpreted on a common scale. We also computed an odds ratio for each covariate by using its unstandardized coefficient, e.g., raising the coefficient to base "e" or $e^{\hat{\beta}_1}$, to facilitate interpretation of the magnitude of its effect on parr densities. As given, these odds ratios are based on a single unit change, whereas larger (or smaller) units of change may be more ecologically interpretable. Therefore, we multiplied relevant unstandardized coefficients by a constant (C) whose magnitude reflected a more meaningful interpretation than a single unit change (e.g., $e^{C\hat{\beta}_1}$; Hosmer and Lemeshow 1989). We obtained an initial estimate of the magnitude of the constant for each covariate based on the difference represented in two standard deviations from its mean as computed from the database compiled by the interior Columbia Basin assessment (Lee et al. 1997). Then, we consulted with subject area experts familiar with the study area to fine-tune these estimates. For example, the model coefficient for percentage of subwatershed containing >50% slopes (Slope) was multiplied by 10 because a 10% change in Slope from one subwatershed to another had more meaning, in terms of physical processes potentially affecting the streams and fish therein, than a single unit (1%) change in Slope. However, we also present unstandardized coeffi-

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Table 2. Model selection results for logistic regression models containing landscape habitat predictor variables and count categories of chinook salmon parr sampled during 1987 (n = 37 subwatersheds (72 stream sections)).

Candidate model	QAICc	ΔQAICc	ΔQAICc weight	% of maximum ΔQAICc weight
Precip, Slope, Georoad	46.50	0	0.379	100
Precip, Mafic	48.48	1.98	0.141	37.2
Precip	48.77	2.27	0.122	32.2
Precip, Slope, Mafic	49.15	2.65	0.101	26.6
Precip, Slope, Unconsol, Georoad	49.16	2.66	0.101	26.6
Precip, Unconsol	50.07	3.57	0.064	16.9
Georoad	51.72	5.22	0.028	7.4
Precip, Slope, Unconsol	51.98	5.48	0.024	6.3
Sumtemp	53.50	7.00	0.011	2.9
Slope, Mafic	53.87	7.37	0.010	2.6
Sumtemp, Mafic	54.19	7.69	0.008	2.1
Global Model	54.94	8.44	0.006	1.6
Slope, Georoad, Mngclus	56.87	10.37	0.002	0.5
Mngclus	58.05	11.55	0.001	0.3
Slope, Mngclus	58.44	11.94	0.001	0.3
Georoad, Mngclus	58.60	12.10	0.001	0.3
Unconsol, Georoad, Mngclus	61.42	14.92	<0.001	<0.1

cients and their standard errors for those interested in interpreting odds ratios based on a single unit change.

We did not simply rely on statistical significance to interpret model results because an odds ratio could be small enough to be considered ecologically unimportant but still be statistically significant (Yoccoz 1991). Note that statistical significance can be construed if the confidence interval for an odds ratio does not include 1; this is equivalent to testing, say, $\beta_1 = 0$, which can be respecified in terms of an odds ratio, $e^{\beta_1} = e^0 = 1$. We evaluated ecological importance of each covariate in the composite model by computing 90% confidence intervals for the scaled odds ratios (e.g., $e^{C\beta \pm 1.64C\widehat{SE}(\beta 1)}$, where $z_{0.95}$ = 1.64; Hosmer and Lemeshow 1989) and interpreting magnitudes of the values contained within these intervals (Gerard et al. 1998). A confidence interval that only contained values whose sizes were considered meaningful indicated an ecologically important relationship between the covariate and parr count categories. Conversely, an interval that only contained values whose magnitudes were considered of minimal importance indicated a covariate exhibiting a weak relationship with parr count categories. Finally, a confidence interval that contained values for odds ratios either on both sides of 1 or whose range included both ecologically important and unimportant magnitudes indicated inconclusive results due to imprecision from inadequate sample sizes.

We computed the predicted probability ($\hat{p}$) that a subwatershed had a low count category of parr (category 1) or a moderate to high count category of parr (category 2) using the formula $\hat{p} = \frac{1}{1 + e^{(-\beta_0 - \vec{\beta}\vec{X})}}$, where β_0 is the model intercept, $\vec{B}$ is the vector of slope estimates, and $\vec{X}$ is the vector of predictor variables (Hosmer and Lemeshow 1989). A Pearson correlation (r) was then calculated between predicted probability and predictor variable(s) with an ecologically important relationship with parr count category in both the chinook salmon and the steelhead composite models. If more than one predictor variable was ecologically important, we used the additional predictors as a basis for stratification for the correlation analysis. For instance, if composite model results indicated that both geometric mean road density and mean annual precipitation had ecologically important relationships with chinook salmon parr count categories, correlations were computed between predicted probability and mean annual precipitation for subwatersheds with both low and medium to high geometric mean road densities.

The SAS statistical package (SAS Institute Inc. 1996) was used for all of our analyses. Both the type I error rate (α) for GOF tests and the confidence coefficient for confidence intervals were set at 0.10 prior to analyses.

Results

We used data from 1987 for chinook salmon and from 1990 for steelhead because these years contained both the highest average parr counts per unit area and the narrowest confidence intervals of these estimates for each species. After removing one obvious outlier whose inclusion caused a serious model lack of fit, the global model for chinook salmon adequately fitted the data (Hosmer–Lemeshow GOF statistic = 6.06, 7 df, P = 0.53). The global model for steelhead also adequately fitted the data (Hosmer–Lemeshow GOF statistic = 9.81, 7 df, P = 0.20) and had no obvious outliers.

For the chinook salmon parr data, the candidate model containing mean annual precipitation, percentage of subwatershed containing >50% slopes, and geometric mean road density was nearly three times more plausible than the next best approximating model (Table 2). The composite habitat model for the chinook salmon data contained three covariates that were statistically significant, two of which had a fairly strong relationship with parr count categories (Table 3). Geometric mean road density exhibited a negative relationship with chinook salmon parr count categories in that moderate to high counts of parr were 1.33 (1/0.750) times less likely to occur in subwatersheds with every increase in 1 km·km^{-2} road densities. Conversely, moderate to high counts of chinook salmon parr were at least 1.29 times more likely to occur in subwatersheds with every 200-mm increase in mean annual precipitation. The lower bound of the odds ratio for percent slope >50% in a subwatershed was statistically significant but of trivial magnitude (Table 3).

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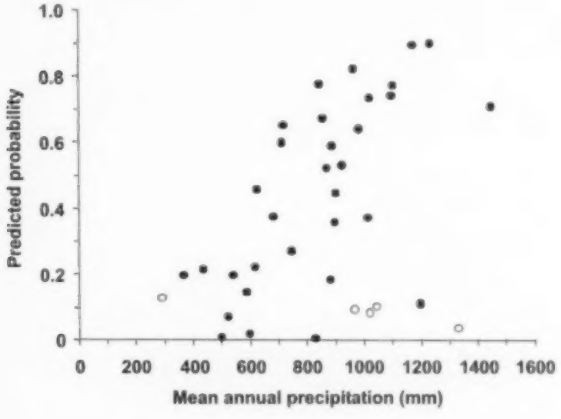
Table 3. Model-averaged results of composite models for chinook salmon and steelhead.

Species	Model parameter	Estimated coefficient (SE)	Standardized coefficient	OR unit change	Estimated OR	90% CI for OR	
						Lower	Upper
Chinook salmon	Intercept	–2.336 (1.531)	—	—	—	—	—
	Precip	0.004 (0.002)	0.658	200	2.164	1.293	3.622
	Slope	–0.125 (0.073)	–0.598	10	0.286	0.086	0.948
	Mafic	–1.490 (1.114)	–0.512	10	<0.001	<0.001	28.992
	Unconsol	0.008 (0.024)	0.079	10	1.080	0.723	1.612
	Georoad	–1.023 (0.448)	–0.624	1	0.360	0.172	0.750
Steelhead	Intercept	–1.357 (1.728)	—	—	—	—	—
	Precip	0.002 (0.001)	0.300	200	1.350	0.922	1.975
	Sumtemp	0.274 (0.140)	0.259	2	1.728	1.092	2.735
	Slope	–0.018 (0.028)	–0.085	10	0.833	0.525	1.324
	Mafic	0.026 (0.009)	0.390	10	1.300	1.120	1.509
	Unconsol	–0.107 (0.044)	–0.645	10	0.342	0.167	0.701
	Georoad	0.191 (0.250)	0.131	1	1.210	0.803	1.824

Note: Model parameters whose 90% confidence intervals (CI) for their estimated odds ratios (OR) do not include 1 are statistically significant.

Fig. 2. Relationship between mean annual precipitation and geometric mean road density (open circles indicate low density and solid circles indicate medium to high density) and predicted probability ($\hat{p}$) of a subwatershed containing either low (e.g., $\hat{p} \leq 0.5$) or moderate to high (e.g., $\hat{p} > 0.5$) count categories of chinook salmon parr.



Evidence was inconclusive for the remaining covariates in the composite habitat model for chinook salmon.

Subwatersheds with low geometric mean road density (<1 km·km^{-2}; adapted from Lee et al. 1997) had a strong positive correlation ($r = 0.643$, $n = 32$) between mean annual precipitation and predicted probability of chinook salmon parr count category, whereas those with medium to high road density (≥1 km·km^{-2}) had a very strong negative correlation ($r = –0.874$, $n = 5$) between these variables. The five subwatersheds containing medium to high geometric mean road densities had predicted probabilities close to 0, which indicated that these subwatersheds were classified as containing low count categories of chinook salmon parr regardless of mean annual precipitation levels (Fig. 2).

For the steelhead parr data, the model containing mean annual precipitation and percent unconsolidated lithology was the best approximating model but was only slightly more plausible than the next highest ranked model (Table 4). The composite habitat model contained three covariates whose odds ratios were statistically significant (Table 3) but only one (percent unconsolidated lithology) had a fairly strong relationship with parr count categories. That is, moderate to high counts of steelhead parr were at least 1.43 (1/0.701) times less likely to occur in subwatersheds with every 10% increase in unconsolidated lithology than low densities. Thus, there was a negative relationship between steelhead parr counts and unconsolidated lithology.

Both mean annual maximum summer temperature and percent mafic lithology had a small positive relationship with moderate to high counts of steelhead parr. Moderate to high steelhead parr counts were at least 1.09 times more likely to occur in subwatersheds with every increase in 2°C mean annual maximum summer temperature and at least 1.12 times more likely to occur in subwatersheds with every increase in 10% mafic lithology (Table 3). Information on all other covariates in the composite habitat model was inconclusive.

Subwatersheds with <10% unconsolidated lithology had a weakly to moderately negative correlation ($r = –0.343$, $n = 69$) with predicted probability of steelhead parr count category, whereas those with >10% unconsolidated lithology exhibited an extremely strong negative correlation ($r = –0.996$, $n = 10$) with these predicted probabilities. Using the typical 0.5 cutoff for categorizing predicted probabilities (Hosmer and Lemeshow 1989), the five subwatersheds with >30% unconsolidated lithology would be classified as containing low count categories of steelhead parr (Fig. 3).

Discussion

Results generated from our analyses must be viewed within the limitations of the parr monitoring and landscape habitat data sets. First, our analyses suffered from the fact that our objective differed from the one originally set forth in the parr monitoring project, and therefore, we subsetted the data accordingly. Second, problems with study design, particularly the unknown impact of bias generated from the nonrandom selection of stream sections and counts uncor-

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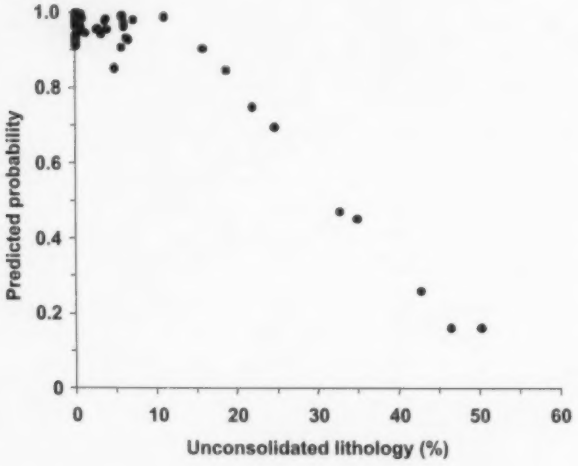
Table 4. Model selection results for logistic regression models containing landscape habitat predictor variables and count categories of steelhead parr sampled during 1990 (n = 79 subwatersheds (155 stream sections)).

Candidate model	QAICc	ΔQAICc	ΔQAICc weight	% of maximum ΔQAICc weight
Precip, Unconsol	81.51	0	0.280	100
Precip, Mafic	82.10	0.59	0.208	74.3
Precip, Slope, Unconsol	82.96	1.45	0.136	48.6
Sumtemp, Mafic	83.23	1.72	0.119	42.5
Precip, Slope, Mafic	84.28	2.77	0.070	25.0
Precip, Slope, Unconsol, Georoad	84.90	3.39	0.051	18.2
Unconsol, Georoad, Mngclus	85.76	4.25	0.034	12.1
Slope, Mafic	85.82	4.31	0.033	11.8
Sumtemp	86.39	4.88	0.024	8.6
Georoad	87.05	5.54	0.018	6.4
Precip	88.30	6.79	0.009	3.2
Global Model	88.58	7.07	0.008	2.9
Precip, Slope, Georoad	89.44	7.93	0.005	1.8
Mngclus	91.89	10.38	0.002	0.7
Georoad, Mngclus	92.76	11.25	0.001	0.4
Slope, Mngclus	94.13	12.62	<0.001	0.2
Sumtemp, Mngclus	94.73	13.22	<0.001	0.1

Fig. 3. Relationship between percent unconsolidated lithology and predicted probability ($\hat{p}$) of a subwatershed containing either low (e.g., $\hat{p} \leq 0.5$) or moderate to high (e.g., $\hat{p} > 0.5$) count categories of steelhead parr.



rected for incomplete detectability of individuals within sections, compelled us to further subset and pool the data. In the latter case, simply modeling raw counts with covariates thought to influence detectability of fish within sampled sections will not correct for sampling bias but will only reflect how well the covariates relate to the biased counts. The matter of confounding still exists. Such a modeling approach would only be valid if (*i*) the nature and magnitude of the counting bias were known for single or repeated counts or (*ii*) repeated counts were conducted on each stream section and the true abundance did not change among counts. Changes in both abundance and covariate values across repeated counts produce confounding between biased counts and covariates. Third, by scaling up to the subwatershed level, we assumed that sampled stream sections were an adequate representation of chinook salmon or steelhead populations for all relevant stream sections within their respective subwatersheds.

Because of various difficulties inherent in the data, in this paper we placed as much emphasis on our analytic approach as we did on interpretation of results. Our procedure for subsetting and modeling a problematic data set should be of interest to fishery biologists, especially because snorkel counts are so commonly used in stream fish studies. We stress, however, that there is no substitute for proper study design and statistically sound sampling methods. It is more preferable to model counts directly than to lose information by pooling data. Nonetheless, we deemed the potential for spurious results due to biased counts to be far more serious than loss of information due to pooling data.

The modeling component of our analyses, in particular, has applications well beyond those used in this paper. AIC-based model selection has a strong theoretical basis (for details, see Burnham and Anderson 1998) and, as such, represents a fundamental departure from traditional methods of model building and variable selection based on null hypothesis testing (e.g., various stepwise and all subset selection procedures). Further, model averaging explicitly incorporates model selection uncertainty into model parameter estimates and also provides a statistically rigorous means to handle the common situation where there is no single model that is clearly better than other models. Ideally, construction of the global and candidate set of models would occur during the design stage of a study and be dictated by the research or management questions being addressed as well as existing information from previous studies. It is important to remember that AIC-based model selection will only choose the best approximating model in the candidate set; it will not correct for poor data or model choice. No analytical methods exist that can completely rescue a data set generated from an inadequately designed study.

Within the boundaries of inference allowed by the data

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set, there were some notable patterns that emerged between parr count categories and various landscape attributes. For instance, the negative relationship between geometric mean road density and count categories of chinook salmon may be of particular interest to land managers who are charged with ensuring the persistence of anadromous salmonid populations. Particular attention should be paid to those subwatersheds with >1 km·km^{-2} geometric mean road densities. Lee et al. (1997) also reported a negative relationship between road densities and fish population status in the Columbia Basin. Unfortunately, the correlative nature of the data is insufficient for identifying the important drivers behind this relationship. Nevertheless, these findings are noteworthy with respect to the recent road closure policy proposed by the USDA Forest Service (Federal Register 1998*b*).

The fairly strong positive influence of mean annual precipitation on count categories of chinook salmon parr may be related to the positive impact that stream discharge typically has on survival rates of anadromous salmonids (Gibson and Myers 1988; Bradford 1994; Fukushima and Smoker 1997). However, other factors related to high stream flows may be influencing chinook salmon parr numbers as well, such as lower predation rates (Bradford 1994), increased rearing habitat (Bradford 1994), and decreased egg mortality due to freezing (Gibson and Myers 1988).

Model results also infer that surrounding lithology may be especially important to steelhead parr numbers, even on a landscape scale. The fairly strong negative relationship between unconsolidated lithology and steelhead parr count categories could be related to sedimentation. An unconsolidated lithology is one that tends to slough off more than other more consolidated lithologies and hence would contribute more sediment inputs into surrounding streams, which could adversely affect parr survival (Crouse et al. 1981; Waters 1995). Conversely, a mafic lithology contains a strong alkaline component, and hence, its inputs may be tied to higher alkalinity in streams, which has been previously related to increased fish productivity (Scarnecchia and Bergersen 1987; Waters et al. 1993; Kwak and Waters 1997). This idea is consistent with the positive relationship between average maximum summer temperature (which was within the range of tolerance for steelhead) and steelhead parr count categories, where elevated summer temperature may increase primary production in a stream or parr metabolism and growth rates.

Our composite model results represent an initial approximation for fishery biologists and managers interested in mapping approximate status and quality of rearing habitats for chinook salmon and steelhead in relevant areas of Idaho. Assuming that our count categories provide an adequate index of density, subwatersheds with medium to high (>1 km·km^{-2}) geometric mean road densities and (or) low (<700 mm) mean annual precipitation levels may indicate low densities of chinook salmon parr, whereas subwatersheds with high percentages (>30%) of unconsolidated lithologies may indicate low densities of steelhead parr. These models could be updated and refined as more and better information became available and then used to help evaluate possible factors affecting salmonid population status and trends. If additional population data are collected, an effort should be made to collect them at the same spatial scale as the predictor variables.

There are probably factors unrelated to habitat that may be affecting status and distribution of these two species in Idaho. For example, deleterious effects of dams on access to spawning and rearing areas, stock productivity, and survival rates could be the overriding factors influencing parr numbers (or even presence) (Schaller et al. 1999). A number of subwatersheds may have an inherent capacity to support high parr densities, based on landscape-level habitat attributes, but may lack proper access for anadromous salmonids (e.g., blockage of the upper Snake River drainage by Hells Canyon Dam). In any event, identifying cause and effect relationships between anthropogenic variables (e.g., road density, land management practices, and dams) and parr numbers will require carefully planned, well-funded, large-scale field experiments.

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Genotypic variation among *Gammarus fasciatus* (Crustacea: Amphipoda) from the Great Lakes – St. Lawrence River: implications for the conservation of widespread freshwater invertebrates

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Abstract: We examined the population genetic structure of the amphipod *Gammarus fasciatus* (Crustacea: Amphipoda) over two years from a total of 11 sites within the Great Lakes – St. Lawrence River system using allozyme electrophoresis. In 1995, we examined differences within the St. Lawrence River (Cornwall to Quebec City), and in 1996, we focused on larger scale, whole-system differences from Lake Superior to Lake Champlain. We found very low levels of genetic differentiation among sites (Wright's $F_{ST} < 0.03$). This was not unexpected for an active disperser in a relatively contiguous system. However, we also found low levels of genetic variability within sites ($H_{obs} = 0.07$- 0.15), a result that was not anticipated. We surveyed the available literature on gammarid and other amphipods and found that limited genetic variability is characteristic of continuously distributed taxa, contrary to the expectations of current conservation theory. This is potentially of concern, especially in light of recently reported declines of *G. fasciatus* at several locations within the St. Lawrence River and Great Lakes. Given the importance of species such as *G. fasciatus* in aquatic food webs, further work is clearly needed on the consequences of population genetic structure to species' responses to novel environmental changes (e.g., exotic species introductions).

Résumé : A l'aide de la technique par électrophorèse enzymatique, nous avons étudié la structure génétique des populations de l'amphipode *Gammarus fasciatus* (Crustacea: Amphipoda) au cours de deux années à 11 sites dans le bassin Grands Lacs – Saint-Laurent. En 1995, l'étude a porté sur les variations le long du corridor fluvial Saint-Laurent entre Cornwall et Québec alors qu'en 1996, l'analyse fut réalisée à une plus échelle spatiale couvrant l'ensemble du bassin depuis le lac Supérieur jusqu'au lac Champlain. Les niveaux de différentiation génétique entre les sites étaient très faibles (indice F_{ST} de Wright <0,03). Ce résultat n'était pas inattendu compte tenu qu'il s'agit d'une espèce à dispersion passive distribuée à l'intérieur d'un système relativement continu. Par contre, la variabilité à l'intérieur des sites était aussi faible ($H_{obs} = 0,07–0,15$), ce qui représente un résultat inattendu. A partir d'une revue de littérature des amphipodes Gammaridae et autres amphipodes, il appert qu'une variabilité génétique faible serait caractéristique des taxa à distribution continue, ce qui est contraire aux prévisions de l'actuelle théorie de la conservation des espèces. Ceci apparaît particulièrement important dans le contexte des diminutions de *G. fasciatus* récemment observées à plusieurs sites du bassin Grands Lacs – Saint-Laurent. Étant donné l'importance relative des espèces d'invertébrés comme *G. fasciatus* dans les écosystèmes aquatiques, des travaux supplémentaires sur les conséquences d'une variabilité génétique limitée des espèces en réponse aux nouveaux changements environnementaux (e.g., introduction d'espèces exotiques) s'avèrent nécessaires.

Introduction

There is increasing recognition of our inability to accurately assess levels of diversity occurring within and among natural populations, particularly at the species and genetic levels (Daugherty et al. 1990; Savage 1995). It is widely contended that the maintenance of such diversity will facilitate the evolutionary potential of natural systems and populations to respond to changing environmental conditions. Much of the recent concern has focused on the ability of species to respond to rapid and novel environmental changes such as increased temperatures (i.e., global warm-

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ing), anthropogenic alteration of habitats, and competition with introduced exotic species. In an attempt to address these concerns for aquatic systems, considerable attention has focused on the genetic structure of commercially important fish species with a view to defining "evolutionary significant units" (e.g., Gharrett et al. 1994; Nielson 1995). Comparatively little work has focused on invertebrates, particularly common species within larger North American systems (e.g., St. Lawrence River). This is unfortunate, since many of these taxa hold pivotal positions in the food chains supporting the more economically important species.

The North American amphipod *Gammarus fasciatus* (Crustacea: Amphipoda) is an example of such a taxon; it is numerically dominant among benthic habitats within the St. Lawrence River and Great Lakes, and it is a prominent component of aquatic food chains (e.g., Price 1963). Recent declines in *G. fasciatus* have been documented at sites in the Great Lakes (Dermott et al. 1998), prompting concern for the status of this species. It is thought that these declines are the direct result of competition with introduced exotics (e.g., *Echinogammarus ischnus*). However, relatively little is known about the population structure or movement patterns of this species throughout the Great Lakes system. Indeed, its original distribution and presence in the Great Lakes (endemic or introduced endemic) are uncertain (Mills et al. 1993).

Previous studies on the genetic structure of taxa within the Great Lakes suggest the existence of genetically distinct stocks between and within lakes and potentially limited gene flow, even for relatively mobile taxa such as lake trout (*Salvelinus namaycush*) (Krueger and Ihssen 1995), walleye (*Stizostedion vitreum*) (Todd and Haas 1993), and mysid shrimp (Väinölä et al. 1994). By contrast, studies of exotic species have often revealed limited genetic variability among locations (e.g., Stepien et al. 1998).

We initiated this study to determine the level of population substructuring for *G. fasciatus* in the Great Lakes – St. Lawrence River system. Previous work on the genetic structure of other *Gammarus* species has shown that populations tend to be highly differentiated, even on small spatial scales (<100 km; e.g., Gooch 1990), indicating low levels of movement and gene flow between populations. However, most previous studies considered noncontinuous (spatially isolated) sites; few studies considered gammarids from relatively continuous habitats. Accordingly, we focused on the genetic structure of *G. fasciatus* populations both within the St. Lawrence River and throughout the Great Lakes system. Our specific goal was to test whether levels of genetic differentiation for *G. fasciatus* among locations in the Great Lakes – St. Lawrence River system would be lower relative to those of gammarid and other amphipod taxa from more discontinuous habitats. Understanding the degree of genetic differentiation among populations of *G. fasciatus* could provide important information for the designation of evolutionary significant units or conservation management units (e.g., Nielson 1995) in the Great Lakes – St. Lawrence River system. Moreover, because genetic data are not currently available for *G. fasciatus*, these data also provide an important baseline for long-term monitoring of *G. fasciatus* populations.

To more broadly examine patterns of population genetic structure in other amphipod taxa, we also surveyed the literature for studies on the genetic structure of gammarid and nongammarid species. These comparisons allowed us to test the hypotheses that species occupying discontinuous habitats would exhibit (*i*) greater levels of genetic differentiation among populations and (*ii*) lower levels of genetic variation within populations relative to species occupying continuous habitats. As we show, some of our findings are counterintuitive and could have important implications for the conservation of freshwater gammarids in the Great Lakes – St. Lawrence River and other freshwater systems.

Methods

Study sites and collection of *G. fasciatus*

The Great Lakes and St. Lawrence River form one of the largest freshwater systems in the world, running 3000 km from Lake Superior in central North America to the Gulf of St. Lawrence in eastern Canada. The system is relatively contiguous, with a network of canals and locks being constructed from the late 1700s through to the 1950s allowing navigation throughout. Perhaps most notable is the Welland Canal, completed in 1919, which provides a navigable link between Lakes Ontario and Erie. In the past, these structures were thought to be responsible for the movement of sea lamprey (*Petromyzon marinus*) into upstream lakes (Mills et al. 1993) and more recently for the spread of exotic species (e.g., *Dreissena* spp., *Echinogammarus* sp.; Hebert et al. 1989; Witt et al. 1997). It is also possible that these navigational channels may have facilitated the movement of *G. fasciatus* throughout the Great Lakes – St. Lawrence River system relative to its other known habitats in eastern North America (e.g., Chesapeake, Hudson, and Delaware rivers), although this remains unclear (Mills et al. 1993).

Individuals of *G. fasciatus* were collected in June 1995 and June 1996 from a series of locations within the Great Lakes – St. Lawrence River system ranging from Lake Superior in the west to Quebec City in the east (Fig. 1). Two sites were within the Great Lakes (Lakes Superior and Ontario), one in a headwater lake (Lake Champlain), and the remainder in fluvial lakes and channels of the St. Lawrence River (Fig. 1). The locations were considered to be hydrologically continuous, as all are connected by navigable channels through the Great Lakes – St. Lawrence River system. Sites were numbered sequentially, with progressively greater integers representing sites further downstream. In 1995, we focused on differences within the St. Lawrence River system. In 1996, we evaluated genetic differences throughout the whole system by recollecting individuals from three sites within the St. Lawrence River (Lac Saint-Laurent, Lac Saint-Pierre, and Quebec City) and comparing them with individuals collected from Lakes Superior, Ontario, and Champlain (Fig. 1).

Genetic analyses

All individuals were morphologically confirmed as *G. fasciatus* using Pennak (1989) and were stored at –80°C until needed for tissue preparation. Individuals were homogenized in 10 μL of a Tris–HCl (pH 8.0) buffer solution and analysed using cellulose acetate electrophoresis (Hebert and Beaton 1989). In the first year of the study, we selected five enzymes that had scorable activity at one or more presumed loci each: aldehyde oxidase (AO; EC 1.2.3.1) (two loci), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), malate dehydrogenase (MDH, EC 1.1.1.37) (two loci), mannose phosphate isomerase (MPI; EC 5.3.1.8), and phosphoglucomutase (PGM; EC 2.7.5.1). In the second year, we added four additional enzyme systems: arginine kinase (ARK; EC 2.7.3.3), glucose-6-dehydrogenase (G6PDH; EC 1.1.1.49), lactate dehydrogenase (LDH; EC 1.1.1.27), and peptidase (PEP; EC 3.4.11/13). One locus (AO-1)

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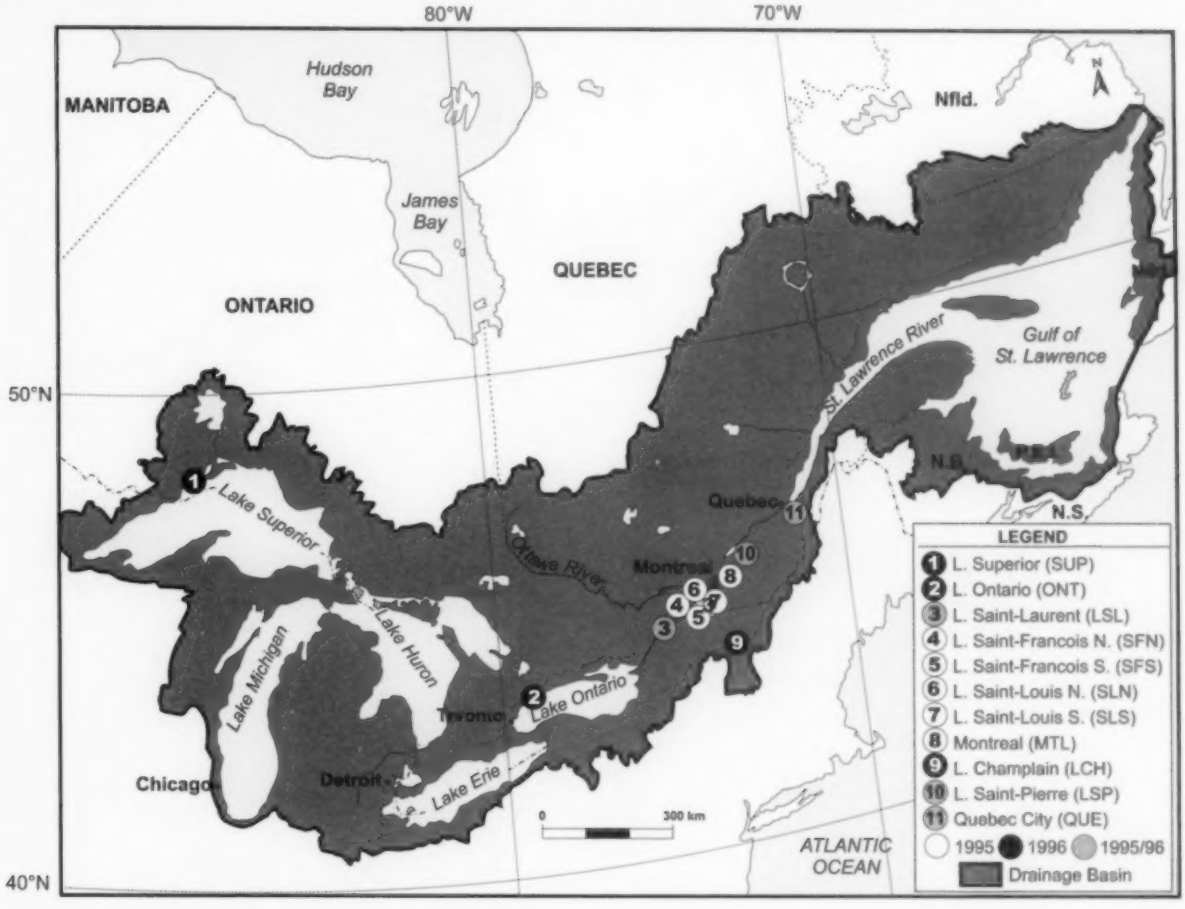
Fig. 1. Study area showing the sites used for the collection of *G. fasciatus* in 1995, 1996, and both years of the study.



could not be reliably scored in the second year of the study due to a change in running conditions and was removed from further analysis.

The fastest alleles (i.e., those migrating closest to the anode) were designated as A, and sequentially slower migrating alleles were designated as B, C, D, etc. To minimize potential scoring errors, we reran at least three individuals from previous gels in each new run and employed gel "lineups" (sensu Richardson et al. 1986) to compare the relative mobility of alleles among sites. In this way, 30% of the individuals analysed during this study were evaluated at least twice. For each locus, we calculated (*i*) the frequency of alleles at each location, (*ii*) agreement of genotypes with Hardy–Weinberg equilibrium (exact probability method), (*iii*) Wright's (1978) F_{ST}, the coefficient of differentiation among populations, (*iv*) genetic distance (Nei 1978), and (*v*) H_{obs}/H_{exp} the proportion of heterozygotes observed/expected (based on Hardy–Weinberg equilibrium) within each site. Measures of genetic variability (i.e., mean number of alleles, percentage of polymorphic loci, H_{obs}/H_{exp}) were calculated in the first instance using sites from the second year of the study only, owing to larger sample sizes and the greater number of loci analysed. However, to facilitate a comparison both among sites and between years, data were then reanalysed by including only those loci that were common between years (AO-1, GPI-1, MPI-1, PGM-1). All calculations were performed using BIOSYS-1 (version 1.7) (Swofford and Selander 1981).

Analysis of data from the literature

We surveyed a variety of bibliographies, reference databases (e.g., Biosys, Ovid), and reference sections of retrieved articles for studies on the population genetic structure of freshwater gammarids and other amphipods. We considered only species for which information on intraspecific population differences and within-population variability (specifically the number of polymorphic loci) was available. Some studies analysed only one or a few loci, which may provide an incomplete assessment of the degree of genetic variation or differentiation present. To compromise between having sufficient numbers of studies for comparison and ensuring that several loci had been surveyed, we included studies providing information from at least five allozyme loci, yielding a total of 29 studies (including the present study). This analysis expands a previous review in which we considered marine, estuarine, and freshwater amphipods from throughout the world (Hogg et al. 1999). Here, we focus only on freshwater amphipods (including several new species not listed by Hogg et al. 1999), and we consider separately *Gammarus* and nongammarid species.

For each study, we recorded information on the habitat type in which the species or population occurred (continuous versus discrete), the degree of genetic differentiation, and the level of genetic variation. The degree of genetic differentiation among locations or populations was most commonly measured as Nei's (1972) genetic

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Table 1. Allele frequencies for polymorphic allozyme loci for *G. fasciatus* collected from sites in the Great Lakes – St. Lawrence River.

		Site													
		1	2	3	3	4	5	6	7	8	9	10	10	11	11
		SUP	ONT	LSL	LSL	SFN	SFS	SLN	SLS	MTL	LCH	LSP	LSP	QUE	QUE
		1996	1996	1995	1996	1995	1995	1995	1995	1995	1996	1995	1996	1995	1996
Locus	Allele	(60)	(60)	(46)	(61)	(43)	(38)	(46)	(38)	(48)	(60)	(43)	(57)	(34)	(55)
AO-1	A			0.19		0.15	0.13	0.14	0.13	0.01		0.06		0.12	
	B			0.26		0.28	0.29	0.25	0.29	0.35		0.24		0.25	
	C			0.20		0.33	0.24	0.33	0.21	0.28		0.30		0.25	
	D			0.26		0.22	0.22	0.22	0.26	0.21		0.29		0.24	
	E			0.08		0.02	0.11	0.07	0.11	0.15		0.11		0.15	
AO-2	A	0.67	0.53	0.55	0.59	0.56	0.55	0.61	0.58	0.63	0.69	0.52	0.44	0.69	0.64
	B	0.33	0.47	0.45	0.41	0.44	0.45	0.39	0.42	0.37	0.31	0.48	0.56	0.31	0.36
G6PDH	A	0.06	0.05		0.04						0.03		0.10		0.06
	B	0.93	0.94		0.91						0.81		0.84		0.90
	C	0.01	0.01		0.05						0.17		0.06		0.04
GPI	A			0.04	0.02	0.01	0.05		0.07		0.02		0.01		0.02
	B				0.01	0.04	0.02	0.04	0.03		0.02		0.03		0.02
	C	1.00	1.00	0.93	0.97	0.94	0.92	0.96	0.91	1.00	0.94	1.00	0.96	1.00	0.96
	D			0.02		0.01					0.02		0.01		
MPI	A										0.02				0.01
	B	0.10	0.08	0.02	0.03	0.01	0.10	0.01	0.05	0.02	0.12	0.02	0.02	0.09	0.06
	C	0.90	0.92	0.94	0.97	0.98	0.82	0.99	0.95	0.96	0.86	0.95	0.98	0.91	0.91
	D			0.03		0.01	0.08			0.02	0.01	0.02			0.03
PEP	A		0.01		0.01										
	B		0.02												
	C	1.00	0.98		0.99						1.00		0.98		1.00
	D												0.02		
PGM	A		0.01	0.01	0.12		0.01	0.01	0.01		0.01	0.05	0.03	0.03	0.04
	B	1.00	0.98	0.96	0.86	0.96	0.91	0.96	0.97	0.97	0.98	0.94	0.92	0.97	0.93
	C		0.02	0.03	0.02	0.04	0.08	0.03	0.01	0.03	0.02	0.01	0.05		0.04

Note: See Fig. 1 for site numbers and abbreviations. Collections were made in June 1995 and June 1996. Sample sizes at each location are given in parentheses.

identity (a measure of genetic (allelic) similarity among sites) or Wright's (1978) F_{ST} (a measure of within-site versus among-site variability; Richardson et al. 1986). In our analyses, we considered values of Nei's (1972) genetic identity of >0.85, 0.45–0.85, and <0.45 to represent "low," "moderate," and "great" levels of differentiation among populations, respectively (after Stewart 1993). When Wright's (1978) F_{ST} measures were reported, we considered values of 0–0.05, 0.05–0.15, 0.15–0.25, and >0.25 to represent "low," "moderate," "great," and "very great" levels of differentiation, respectively (after Hartl 1988). Genetic variability within populations was measured as the percentage of polymorphic loci. We treated each study as a single independent data point for our comparisons.

In an initial analysis, we found a negative correlation between the number of loci examined in each study and the percentage of loci that were polymorphic ($r = -0.45$, $n = 29$, $P < 0.02$; percentages of polymorphic loci were arcsin square root transformed prior to analysis). Studies using only a small number of loci appear to have focused on the most polymorphic (and therefore informative) loci, whereas studies examining large numbers of loci (e.g., >10) apparently included additional but less variable loci. Accordingly, to compare among studies with different numbers of loci, we needed to correct for this variation. We did so in two ways: (*i*) we used analysis of covariance, with number of loci as the covariate, to test for main effects of habitat type (continuous versus discrete) and taxonomy (gammarid versus nongammarid) and (*ii*) we calculated the residuals of the regression of the percentage of polymorphic loci (arcsin square root transformed) on the number of loci analysed in each study and used these residuals in all further comparisons. This analysis removes the effect of the number of loci and allows a comparison of the levels of genetic variability among habitat types and taxa. Both sets of analyses produced similar results. We also examined the relationship between the degree of genetic differentiation (low, moderate, great, and very great) and both habitat type and level of polymorphism. Small sample sizes precluded analysis of these relationships for gammarid versus nongammarid amphipods separately. We used residuals of the regression of the percentage of polymorphic loci (arcsin square root transformed) on the number of loci as the dependent variable in these comparisons.

Results

Population genetic structure of *G. fasciatus*

Allele frequencies at each of the sites for variable loci are shown in Table 1. Four loci (ARK, MDH-1, MDH-2, LDH) showed no variation for any individuals collected at any of the sites. A further locus (PEP) showed frequencies of the most common allele at >95% and hence could also be considered fixed. Of the remaining six polymorphic loci (95% criteria), a further three (GPI, MPI, PGM) showed fixation or frequencies of the most common allele >95% at one or more sites (e.g., Lakes Superior and Ontario). Indeed, with the exception of the AO-1 locus, the frequencies of the most

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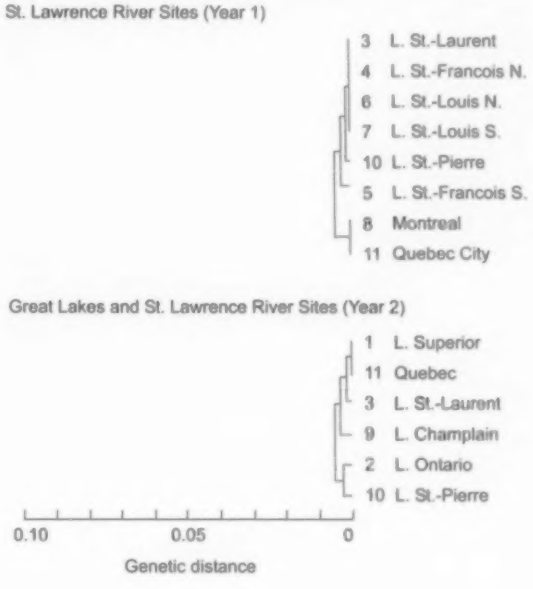
common allele were >80% in all cases (Table 1). Rare alleles were found for individuals collected from several locations, particularly at sites within the St. Lawrence River (e.g., sites 4, 5, 6, 7). However, none was unique to a particular location.

The occurrence and frequencies of alleles for most loci were relatively similar among sites and between years (Table 1). The frequencies of alleles for each of the loci were in accordance with Hardy–Weinberg expectations ($P > 0.05$ in all cases).

Genetic differentiation among locations was low. Wright's (1978) F_{ST} was <0.02 for sites within the St. Lawrence River and only marginally greater (0.03) when considering sites throughout the Great Lakes – St. Lawrence River system. Analyses of genetic distance (Nei 1978) revealed little difference among locations in either of the study years (Fig. 2).

For sites visited in the second year of the study, the number of polymorphic (variable) allozyme loci ranged from 30 to 40% among locations, and the mean number of alleles ranged from 1.4 to 2.1 (Table 2). Overall levels of observed heterozygosity ranged from 0.07 to 0.11 and were comparable among locations. Expected levels of heterozygosity (based on Hardy–Weinberg equilibrium) were in close agreement with observed values, although sites within the Great Lakes (sites 1 and 2) had slightly lower levels relative to the other locations (Table 2). To facilitate comparison among sites between years, we reanalysed the data using only those loci common to both years of the study (AO-1, GPI, MPI, PGM); a similar pattern emerged (Table 3). The highest measures of variability (mean number of alleles, percentage of polymorphic loci, heterozygosity) were recorded on the southern shores the St. Lawrence River and in Lake Champlain (Table 3). For the three sites monitored for each of the two study years, two (Lacs Saint-Laurent and Saint-Pierre) had lower measures of variability in the second year of the study, whereas the other site (St. Lawrence River at Quebec City) had similar measures between years.

Fig. 2. Genetic distance (Nei 1978) among *G. fasciatus* collected from sites in the St. Lawrence River in June 1995 (year 1) and from sites throughout the Great Lakes – St. Lawrence River system in June 1996 (2ear 2). See Fig. 1 for site numbers and locations.



Comparison with other amphipods

Studies of the genetic structure of other freshwater gammarids and nongammarids are summarized in Table 4. Although the number of total observations is somewhat low ($n = 28$ plus the current study), several patterns emerged. As predicted, amphipods occupying discrete or discontinuous habitats exhibited greater levels of genetic differentiation relative to species occupying continuous habitats (small sample sizes precluded analysis of gammarid versus nongammarid species separately). Species in continuous habitats exhibit low ($n = 5$) to moderate ($n = 4$) levels of genetic differentiation, whereas species in discrete habitats exhibit moderate ($n = 13$), great ($n = 2$), or very great ($n = 5$) levels of differentiation (G test with Williams' correction for continuity, $G_w = 11.06$, df = 3, $P = 0.011$) (Table 4); pooling categories of great and very great to increase sample size did not alter this pattern ($G_w = 11.20$, df = 2, $P = 0.004$).

Analyses of genetic variation within and among habitat types and taxa were potentially confounded by the negative relationship between the number of loci considered in each study and the proportion of loci that were polymorphic (see Methods). Analysis of covariance, with number of loci as the covariate, habitat type (continuous versus discrete) and taxon (gammarid versus nongammarid) as main effects, and proportion of polymorphic loci (arcsin square root transformed) as the dependent variable, revealed no significant three-way or two-way interactions (all $F < 1.00$, df = 1,21, $P > 0.34$). When these terms were dropped and only main effects considered, all three variables influenced the level of genetic variation observed among the 29 studies in the survey (number of loci: $F = 3.93$, df = 1,25, $P = 0.058$; taxon: $F = 4.49$, df = 1,25, $P = 0.044$; habitat type: $F = 11.12$, df = 1,25, $P < 0.003$). To further examine these patterns, we removed the effect of number of loci using the residuals of the regression of proportion of polymorphic loci on number of loci surveyed (see methods). Two-way analysis of variance of the effects of habitat type and taxon, using these residuals as the dependent variable, confirmed our analysis. Habitat type had a strong and significant impact on the level of genetic variation ($F = 9.96$, df = 1,26, $P = 0.004$), while there was a smaller and marginally significant difference between gammarid and nongammarid taxa ($F = 4.00$, df = 1,26, $P = 0.056$; the interaction term was not significant and was dropped: $F = 0.01$, df = 1,25, $P > 0.90$). These patterns are illustrated in Fig. 3.

Surprisingly, species in continuous habitats exhibited lower levels of genetic variability within populations compared with species in discontinuous habitats, contrary to our expectations (Fig. 3). This pattern occurred in both gammarid and nongammarid taxa, although gammarids tended to have relatively reduced variation in continuous habitats, whereas nongammarids were relatively more variable in discrete habitats (hence the marginally significant interaction term) (Fig. 3).

The level of genetic variability also varied inversely with

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Table 2. Measures of genetic variability for *G. fasciatus* collected from sites in the Great Lakes – St. Lawrence River in June 1996.

Site	Sample size per locus	No. of alleles	% polymorphic loci	Observed heterozygosity (direct count)	Expected heterozygosity
1. Lake Superior	59.9 (0.1)	1.4 (0.2)	30.0	0.08 (0.05)	0.08 (0.05)
2. Lake Ontario	59.8 (0.2)	1.8 (0.3)	30.0	0.07 (0.04)	0.09 (0.05)
3. Lac Saint-Laurent (1996)	60.9 (0.1)	1.9 (0.3)	30.0	0.08 (0.03)	0.11 (0.05)
9. Lac Champlain	59.8 (0.1)	2.1 (0.4)	40.0	0.11 (0.05)	0.12 (0.05)
10. Lac Saint-Pierre (1996)	56.6 (0.4)	2.0 (0.3)	30.0	0.10 (0.04)	0.11 (0.05)
11. Quebec City (1996)	55.0 (0.0)	2.0 (0.4)	40.0	0.10 (0.04)	0.10 (0.05)

Note: Values are means (±1 SE).

Table 3. Interyear and intersite comparison of measures of genetic variability for *G. fasciatus* collected from sites in the Great Lakes – St. Lawrence River in June 1995 and June 1996.

Site	Sample size per locus	No. of alleles	% polymorphic loci	Observed heterozygosity (direct count)	Expected heterozygosity
1. Lake Superior	59.8 (0.2)	1.3 (0.2)	33.3	0.11 (0.09)	0.11 (0.07)
2. Lake Ontario	59.7 (0.3)	1.7 (0.3)	33.3	0.10 (0.06)	0.12 (0.08)
3. Lac Saint-Laurent (1995)	44.7 (0.8)	2.2 (0.4)	50.0	0.11 (0.06)	0.14 (0.08)
3. Lac Saint-Laurent (1996)	60.8 (0.2)	1.8 (0.3)	16.7	0.07 (0.04)	0.11 (0.08)
4. Lac Saint-Francois north	42.2 (0.3)	2.2 (0.5)	33.3	0.09 (0.05)	0.12 (0.08)
5. Lac Saint-Francois south	44.2 (0.9)	2.2 (0.4)	66.7	0.15 (0.08)	0.19 (0.08)
6. Lac Saint-Louis north	45.2 (0.7)	1.8 (0.3)	16.7	0.12 (0.08)	0.11 (0.08)
7. Lac Saint-Louis south	37.0 (0.8)	2.0 (0.4)	50.0	0.14 (0.08)	0.14 (0.08)
8. Montreal	47.7 (0.2)	1.7 (0.3)	16.7	0.08 (0.06)	0.10 (0.08)
9. Lac Champlain	59.8 (0.2)	2.2 (0.6)	50.0	0.13 (0.07)	0.13 (0.07)
10. Lac Saint-Pierre (1995)	42.5 (0.3)	1.8 (0.4)	33.3	0.11 (0.07)	0.12 (0.08)
10. Lac Saint-Pierre (1996)	56.3 (0.7)	2.0 (0.4)	16.7	0.09 (0.06)	0.11 (0.08)
11. Quebec City (1995)	34.0 (0.0)	1.5 (0.2)	33.3	0.10 (0.06)	0.11 (0.07)
11. Quebec City (1996)	55.0 (0.0)	2.0 (0.5)	33.3	0.11 (0.07)	0.12 (0.08)

Note: Same loci (AO-1, GPI, MPI, PGM) were used for both years to aid in the comparison. Values are means (±1 SE).

the degree of genetic differentiation, contrary to our expectations (analysis of variance of polymorphic loci (residuals) across each category of genetic differentiation: $F = 3.08$, $df = 3.25$, $P = 0.046$) (Fig. 4). Populations with low degrees of differentiation exhibited the fewest polymorphic loci, whereas populations with great levels of differentiation exhibited the most polymorphic loci (Fig. 4). The number of polymorphic loci declined slightly in highly differentiated populations (very great). Due to limited sample size, we could not examine these effects for gammarid and non-gammarid species separately.

Discussion

The response of species to novel environmental perturbations (e.g., climate change, habitat alteration, competition with exotic species) may depend critically on the genetic structure of their populations (Hogg et al. 1998*a*). Our study of *G. fasciatus* in the St. Lawrence River and Great Lakes suggests that genetic variability both within and among populations was limited, particularly at sites within the Great Lakes. Reduced genetic differentiation among populations of *G. fasciatus* was not unexpected, given the relatively continuous nature of the Great Lakes system. However, there was little or no allelic variability for several loci and at several sites, particularly those sites in the Great Lakes (Lakes Superior and Ontario). This limited genetic variability within populations was more surprising.

One possible explanation is that *G. fasciatus* may be a relatively recent introduction into much or all of the Great Lakes and has experienced a range expansion concurrent with the developing network of navigable canals throughout the Great Lakes system. Accordingly, present populations could have been founded by relatively few individuals originating from the St. Lawrence River or other nearby systems (e.g., Hudson, Chesapeake, or Delaware River). There may be some evidence to support this notion, given that habitats in the St. Lawrence River had higher measures of variability relative to sites in the Great Lakes.

However, our results for *G. fasciatus* were similar to those found for other species of freshwater gammarid and non-gammarid amphipods. Measures of variability in *G. fasciatus* (e.g., percentage of polymorphic loci) were low relative to other amphipods collected from discrete habitats (e.g., *Gammarus fossarum*, *Chaetocorophium lucasi*) but were comparable with levels found for taxa collected from more continuous habitats (e.g., *Gammarus caparti*, *Gammarus pulex pulex*, *Brandtia lata*). For example, comparisons of *G. fasciatus* with another species of amphipod (*Hyalella azteca*) collected from discrete habitats within and near the Great Lakes – St. Lawrence River system (Hogg et al. 1998*b*) indicated that measures of variability (e.g., mean number of alleles, levels of heterozygosity) were considerably lower for *G. fasciatus*.

Considering a wider range of amphipod taxa, Hogg et al. (1999) found that lower levels of genetic variability were a

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Table 4. Summary of studies providing data on the intraspecific population genetic structure of freshwater gammarid and nongammarid amphipods.

Species	Habitat type	Differentiation[a]	% polymorphic	Reference
Gammaridae				
Echinogammarus calvus	Discrete (stream/rivers, Europe)	Moderate, D(max) = 0.42	50 (8)	Lop and Oliver 1989
Gammarus caparti	Continuous (lower reaches, streams/rivers, Europe)	Low, I(min) = 0.98	25 (20)	Scheepmaker and van Dalfsen 1989
G. fossarum	Discrete (?) (headwater streams, Europe)	Moderate, I(min) = 0.51	55 (20)	Scheepmaker and van Dalfsen 1989
	Discrete(?) (upper stream reaches, Germany)	Moderate (high)	83 (6)	Siegismund 1988
	Discrete (streams/rivers, Europe)	Moderate, I(min) = 0.63	65 (20)	Scheepmaker 1990*a*
G. gautheri	Discrete (streams/rivers, Europe)	Moderate, I(min) = 0.50	60 (20)	Scheepmaker et al. 1988
	Discrete (streams/rivers, Europe, North Africa)	Great, D(max) = 0.84	85 (20)	Scheepmaker 1990*b*
G. ibercus	Discrete (?) (streams/rivers, Europe)	Moderate, I(min) = 0.70	38 (21)	Scheepmaker et al. 1988
G. minus	Discrete (cave springs, springs within 75 km, eastern North America)	Moderate, I = 0.67	19 (16)	Gooch and Hetrick 1979
	Discrete (streams within 100 km, eastern North America)	Very great, F_{ST} = 0.41	44 (9)	Gooch 1990
	Continuous (streams within same watershed, eastern North America)	Moderate, F_{ST} = 0.09	27 (15)	Gooch 1989
	Discrete (streams within 5 km, eastern North America)	Very great, F_{ST} = 0.37	44 (18)	Kane et al. 1992
	Discrete (cave streams, streams, eastern North America)	Great, F_{ST} = 0.23	53 (19)	Sarbu et al. 1993
G. orinos	Discrete (streams/rivers, Europe)	Moderate, I(min) = 0.67	65 (20)	Scheepmaker 1990*a*
G. pulex pulex	Continuous (lower reaches, streams/rivers, Europe)	Low, I(min) = 0.96	20 (20)	Scheepmaker and van Dalfsen 1989
G. roeseli	Continuous (lower stream reaches, Germany)	Low	33 (6)	Siegismund 1988
G. stupendous	Discrete (?) (streams within 80 km, possible subterrain link, Europe)	Very great, I(min) = 0.12	20 (20)	Scheepmaker 1987
Non-Gammaridae				
Brandtia lata	Continuous (within Lake Baikal)	Low, F_{ST} = 0.04	25 (24)	Yampolsky et al. 1994
Chaetocorophium lucasi	Discrete (lower stream reaches, estuaries, inland lakes, North Island, New Zealand)	Moderate, I(min) = 0.80	82 (11)	Schnabel et al. 1999
Hyalella azteca	Discrete (streams, ponds, lakes within and near the Great Lakes – St. Lawrence River watershed)	Very great, F_{ST} = 0.56	100 (10)	Hogg et al. 1998*b*
	Discrete (spring-fed ponds within 100 km, eastern North America)	Moderate, F_{ST} = 0.129	63 (8)	I.D. Hogg, J.M. Eadie, and D.D. Williams, unpublished data
Paramelita auricularius	Discrete (mountain streams within 500 km, South Africa)	Moderate, I(min) = 0.80	71 (7)	Stewart and Griffiths 1992
P. capensis	Discrete (mountain streams within 440 km, South Africa)	Very great, I(min) = 0.20	100 (10)	Stewart 1992
P. crassicornis	Discrete (mountain streams within 500 km, South Africa)	Moderate, I(min) = 0.63	71 (7)	Stewart and Griffiths 1992
Pontoporeia affinis	Discrete (lakes, northern Europe)	Moderate, F_{ST} = 0.06	37 (19)	Väinölä and Varvio 1989
P. hoyi	Continuous (?) (lakes, within Great Lakes drainage basin)	Moderate, F_{ST} = 0.06	21 (19)	Väinölä and Varvio 1989
Spinacanthus parasiticus	Continuous (within Lake Baikal)	Moderate, F_{ST} = 0.11	33 (24)	Yampolsky et al. 1994
S. armatus	Continuous (within Lake Baikal)	Moderate, F_{ST} = 0.14	38 (24)	

Note: Data included are habitat type (discrete versus continuous), genetic differentiation among locations, and the percentage of polymorphic loci (value in parentheses indicates the number of loci analysed) as a measure of genetic variability.

[a] F_{ST}, Wright's (1978) F_{ST}; I, Nei's (1972) genetic identity; D, Nei's (1972) genetic distance. Designations of low, moderate, and great are based on Wright (1978) for F_{ST} values and Stewart (1993) for I values.

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Fig. 3. Percentage of polymorphic loci (shown here as a residual; see text) as a function of the type of habitat (continuous versus discrete) of freshwater gammarid amphipods (solid bars) and nongammarid (open bars) amphipods. Numbers under the bars represent sample sizes (number of studies). Original sources are listed in Table 4.

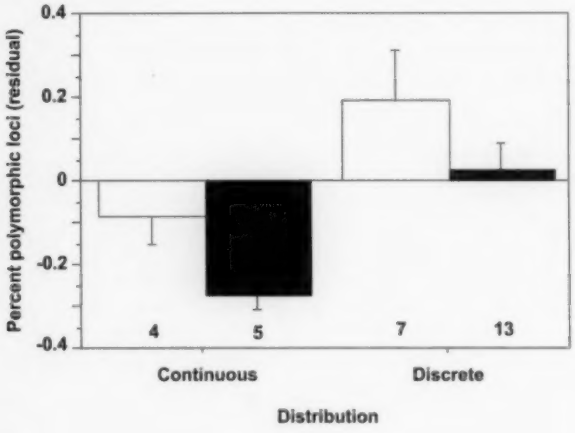
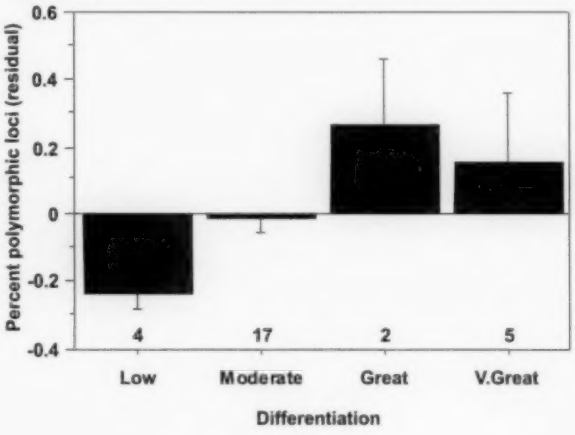


Fig. 4. Percentage of polymorphic loci (residual) as a function of the level of genetic differentiation among populations of freshwater gammarids. Numbers under the bars represent sample sizes (number of studies). Original sources are listed in Table 4.



common feature for taxa collected from relatively continuous habitats compared with similar species in discontinuous habitats. Our survey of freshwater gammarid and nongammarid amphipods is consistent with this pattern, although there were slight differences between taxa. Moreover, both surveys revealed an unexpected relationship: species with greater differentiation among populations exhibited higher, rather than lower, levels of within-population variation.

These results are counter to much current conservation thinking. While it is not unexpected that species in fragmented, discontinuous habitats would exhibit greater levels of differentiation among populations, it is thought that this should be accompanied by reduced levels of genetic variation within populations because of the loss or fixation of alternative alleles in isolated populations through increased genetic drift and reduced gene flow. Accordingly, much conservation effort has focused on maintaining the genetic variability and viability of discrete, isolated populations (Frankham 1995). Our results suggest that the opposite pattern applies to amphipods: rather than exhibiting reduced genetic variability, isolated populations instead exhibit higher levels of variation compared with counterparts in more continuous systems.

The mechanisms underlying such patterns are presently unclear (also see Hogg et al. 1999). One possible explanation is that these patterns reflect an incomplete resolution of freshwater invertebrate taxa. Studies of several amphipod taxa have revealed the presence of morphologically cryptic species (e.g., Hogg et al. 1998*b*). Indeed, of the 20 studies of species in discrete, discontinuous habitats, seven reported evidence of great to very great levels of differentiation (based on Nei's index or Wright's (1978) F_{ST}). Values of this magnitude are often interpreted as indicating distinct subspecies or species status (e.g., Jackson and Resh 1992). Accordingly, the high degree of genetic differentiation observed among amphipods in discrete habitats could indicate the presence of cryptic species. Lumping several cryptic species into a single "population" would result in an inflated estimate of the percentage of polymorphic loci for that population, if these cryptic species differed sufficiently in allelic composition. Populations in discrete habitats would therefore exhibit *both* greater genetic differentiation *and* greater genetic variation (as measured by percentage of polymorphic loci) relative to those in continuous habitats, precisely the pattern that we found. However, this would not explain why there might be more cryptic species *within* discrete habitats in the first place. Moreover, if cryptic species were more likely to be lumped in discrete habitats, we would expect reduced heterozygosity in these "populations" relative to that expected (i.e., a Wahlund effect). This could be assessed directly by comparing observed to expected heterozygosity for these "populations". Unfortunately, too few of the studies in our sample reported these measures to allow us to conduct this analysis. Whatever the mechanism, the high measures of Wright's (1978) F_{ST} observed for many amphipods in discrete habitats indicate that species diversity for freshwater amphipods (and perhaps many other aquatic organisms) may be significantly underestimated, a result that would have important conservation implications. Clearly, patterns of genetic variation among populations need to be considered fully in future efforts to designate evolutionary significant units or conservation management units (e.g., Gharrett et al. 1994; Nielson 1995). Any attempt to develop meaningful conservation strategies for aquatic systems in general, and amphipods in particular, will have to first establish some method to assess existing biodiversity before it will be possible to evaluate any changes in diversity brought about by environmental perturbation.

Our observation that populations in large, continuous habitats (e.g., the Great Lakes – St. Lawrence River system) exhibit reduced genetic variation relative to those in isolated, discrete habitats also indicates that some rethinking is warranted for freshwater amphipods. Specifically, if genetic

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variability within a population is an indication of the effective population size and (or) the ability of that population to adapt to rapid environmental change (e.g., Frankham 1995), it may be that the risk of extinction or extirpation will be greater for populations in continuous habitats (contrary to many current conservation efforts to protect populations in fragmented habitats). Unfortunately, it is precisely these populations that are often exposed to the most severe environmental perturbations (e.g., exotic species introductions, industrial effluents). Clearly, we now need to more fully examine the extent to which the reduced genetic variation observed in several amphipod populations in continuous habitats reflects either small effective population size or a more limited evolutionary potential.

To develop strategies for the conservation of widespread and continuously distributed taxa, we suggest that further work should be targeted towards evaluating levels of genetic differentiation and variability for *G. fasciatus* and other widespread taxa throughout their North American ranges. Laboratory-based studies could then be used to document the responses of different populations to various stresses (e.g., alternative food sources, feeding strategies, contaminant sensitivity). Clearly, the preservation of existing populations is the most desirable option from a conservation perspective. In this regard, habitats on the south shores of the St. Lawrence River appear to contain higher levels of genetic diversity for *G. fasciatus* and may represent the original populations of *G. fasciatus* in the Great Lakes – St. Lawrence River system; populations at these sites should be considered a conservation priority.

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Aggregation and dispersion of krill at channel heads and shelf edges: the dynamics in the Saguenay – St. Lawrence Marine Park

Diane Lavoie, Yvan Simard, and François J. Saucier

Abstract: The spatial organization of the euphausiid (*Thysanoessa raschi* and *Meganyctiphanes norvegica*) aggregation at the head of the Laurentian Channel is examined using 120-kHz echointegration data from eight surveys in the summers of 1994 and 1995 and currents obtained from a high-resolution three-dimensional circulation model. Circulation is the main factor controlling the abundance and distribution of krill. The main aggregation pattern is U-shaped and includes an entrance corridor along the northern edge of the channel, a major accumulation zone off Les Escoumins, and an exit corridor along the southern edge. However, this mesoscale aggregation exhibits rapid fluctuations in spatial pattern and global abundance, due to the redistribution of krill within and out of the study area. The local accumulations are controlled by the interactions between the semidiurnal tidal currents, the topography, and the negative phototactism of krill. The strong vertical currents found along the channel slopes upstream of Les Escoumins and at the sills act to concentrate and pile up krill. The aggregation and dispersion mechanisms are strongly influenced by the deepwater blocking process taking place at the sills. The fortnightly tidal cycle and freshwater runoff modulate the blocking intensity. This krill aggregation dynamics is of primary importance for the trophic link with the baleen whales and fish in the Saguenay – St. Lawrence Marine Park.

Résumé : L'organisation spatiale de l'agrégation d'euphausides (*Thysanoessa raschi* et *Meganyctiphanes norvegica*) à la tête du Chenal Laurentien est examinée à l'aide de données d'échointégration à 120 kHz acquises lors de huit missions aux étés 1994 et 1995 et de courants obtenus d'un modèle de circulation tridimensionnel à haute résolution. La circulation est le principal facteur contrôlant l'abondance et la distribution du krill. Le patron d'agrégation principal a la forme d'un U et inclut un corridor d'entrée le long du rebord nord du chenal, une zone d'accumulation majeure au large des Escoumins et un corridor de sortie le long du rebord sud. Toutefois, le patron spatial et la biomasse de cette agrégation à méso-échelle montrent de rapides fluctuations dues à la redistribution du krill à l'intérieur et à l'extérieur de la zone d'étude. Les accumulations locales sont contrôlées par l'interaction entre les courants de marée semi-diurne, la topographie et le phototactisme négatif du krill. Les forts courants verticaux présents le long des rebords du chenal en amont des Escoumins et aux seuils, concentrent et empilent le krill. Les mécanismes d'agrégation et de dispersion sont fortement influencés par le processus de blocage des eaux profondes se produisant aux seuils. Le cycle de vive-eau – morte-eau et le débit d'eau douce modulent l'intensité du blocage. La dynamique sous-jacente à la formation de cette importante agrégation de krill est d'une importance primordiale dans le lien trophique avec les baleines et les poissons du Parc Marin Saguenay – Saint-Laurent.

Introduction

The combination of the biological behaviour of the euphausiids with persistent horizontal and vertical current systems, such as upwelling, gyres, two-layer circulation, shelf-break, and coastal currents, has been invoked to explain the transport, maintenance, and accumulation of krill in many regions of the world (e.g., Simard and Mackas 1989; Barange and Pillar 1992; Ichii et al. 1998). The mechanism involved is the interaction of the three-dimensional circulation with the vertical distribution and behaviour of euphausiids over a broad continuum of scales, from metres to hundreds of kilometres and from minutes to the whole life cycle of the species. At large scales (>100 km, greater than months), the different vertical distributions of life stages and the diel vertical migration (DVM) of the organisms combine with the current structure to retain the species in a given system (e.g., Barange and Pillar 1992; Mackas et al. 1997) and to generate a zonation with age as observed in the Gulf of St. Lawrence (Berkes 1976) and elsewhere (e.g., Pillar et al. 1989; Ichii et al. 1998). However, local aggregations are generated by meso- (5–100 km, days) or small-scale processes occurring at the site of strong interactions of the deep

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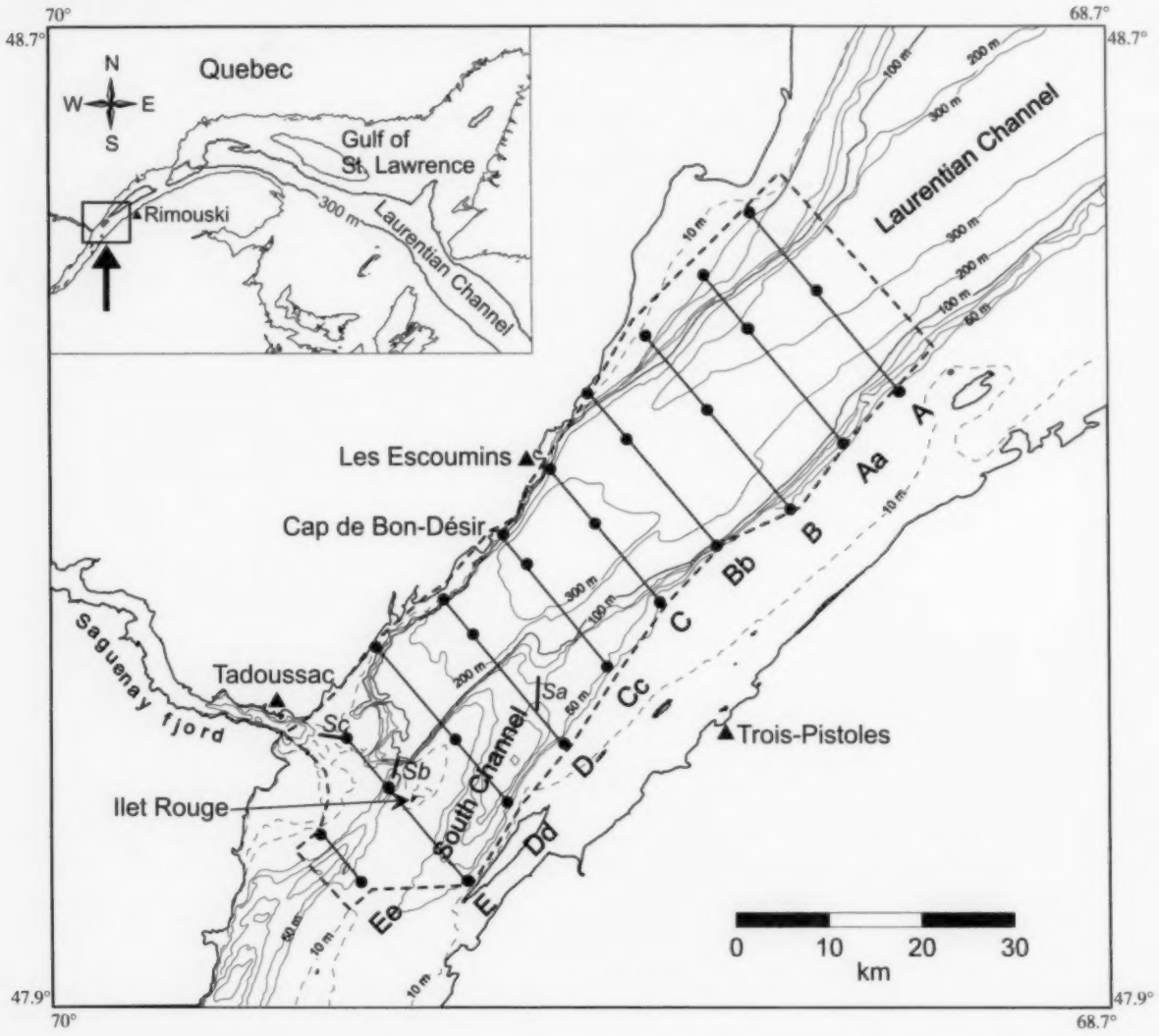
Fig. 1. Map of the study area showing the systematic sampling grid with acoustic transects (lines) and CTD/zooplankton stations (circles). The dashed polygon delimits the 1319-km^2 area used for biomass estimation. The three sills, Sa, Sb, and Sc, bounding the channel head are identified.



circulation with bathymetric features, such as continental or coastal shelf-breaks or edges, deep basins on shelves, and channel or canyon heads, which often correspond to regions of upward-domed isotherms. The head of the Laurentian Channel (LC) in the St. Lawrence Estuary is one such region where the typical two-layer estuarine circulation builds a persistent, but fluctuating, dense mesoscale aggregation (Simard et al. 1986*b*; Simard and Lavoie 1999). This aggregation and its links with the abundance of fish species, especially capelin (*Mallotus villosus*), attracts many species of whales to the area during summer. It is at the base of a large ecotourism industry and of the establishment of the Saguenay – St. Lawrence Marine Park.

The LC is the main channel connecting the St. Lawrence system to the Atlantic (Fig. 1). Its depth varies from ~450 m at the entrance of the Gulf of St. Lawrence to ~325 m near the head of the channel. There, over ~20 km, it shoals to a barrier of banks, islands, and narrow and shallow sills (<40 m) off Tadoussac (sills Sb and Sc, Fig. 1), which separates the Lower Estuary from the Upper Estuary and the Saguenay fjord. Southeast of Ilet Rouge Bank, the South Channel (SC) reaches ~100 m and connects to the LC through a 70-m-deep sill (sill Sa, Fig. 1) off Cap de Bon-Désir. A particular feature of the St. Lawrence system is the presence, from April to December, of a cold intermediate layer (CIL) extending below the surface mixed layer down to over 150 m depth with core temperatures less than 0°C (Banks 1966). At the LC head, the interaction of the flooding barotropic tide (M_2) with the rapidly shoaling bottom leads to strong upwelling of CIL waters over the sills twice

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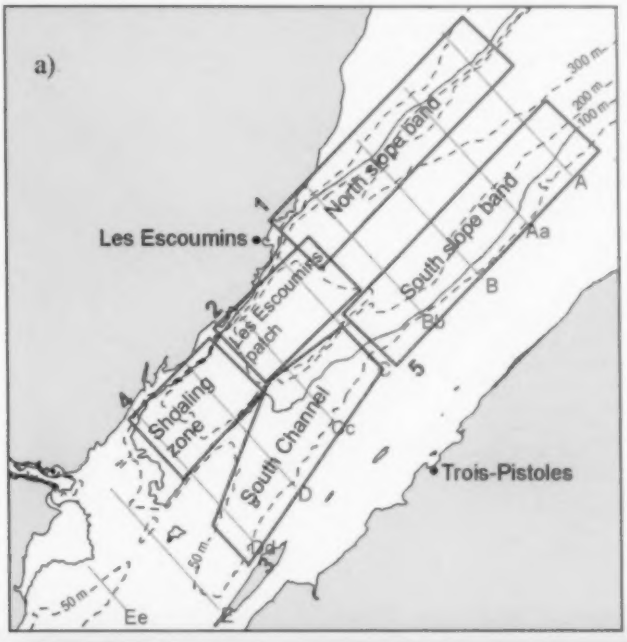
Fig. 2. (*a*) Zones of krill aggregation discussed in the text and (*b*) a diagram of the principal circulation characteristics responsible for the observed aggregations and distribution patterns.



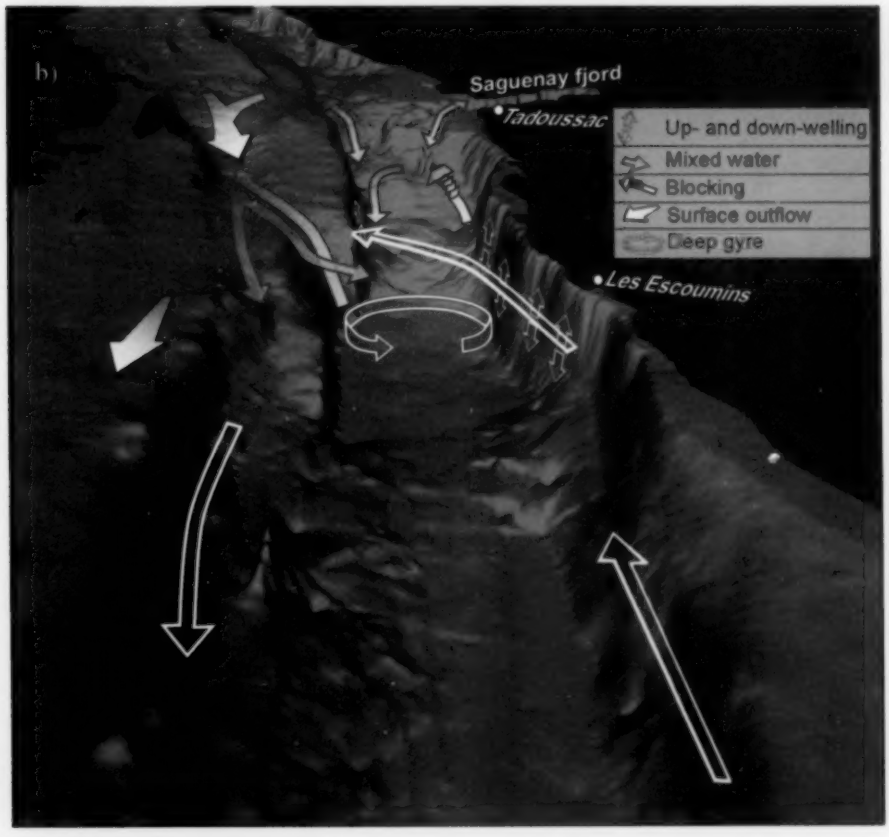


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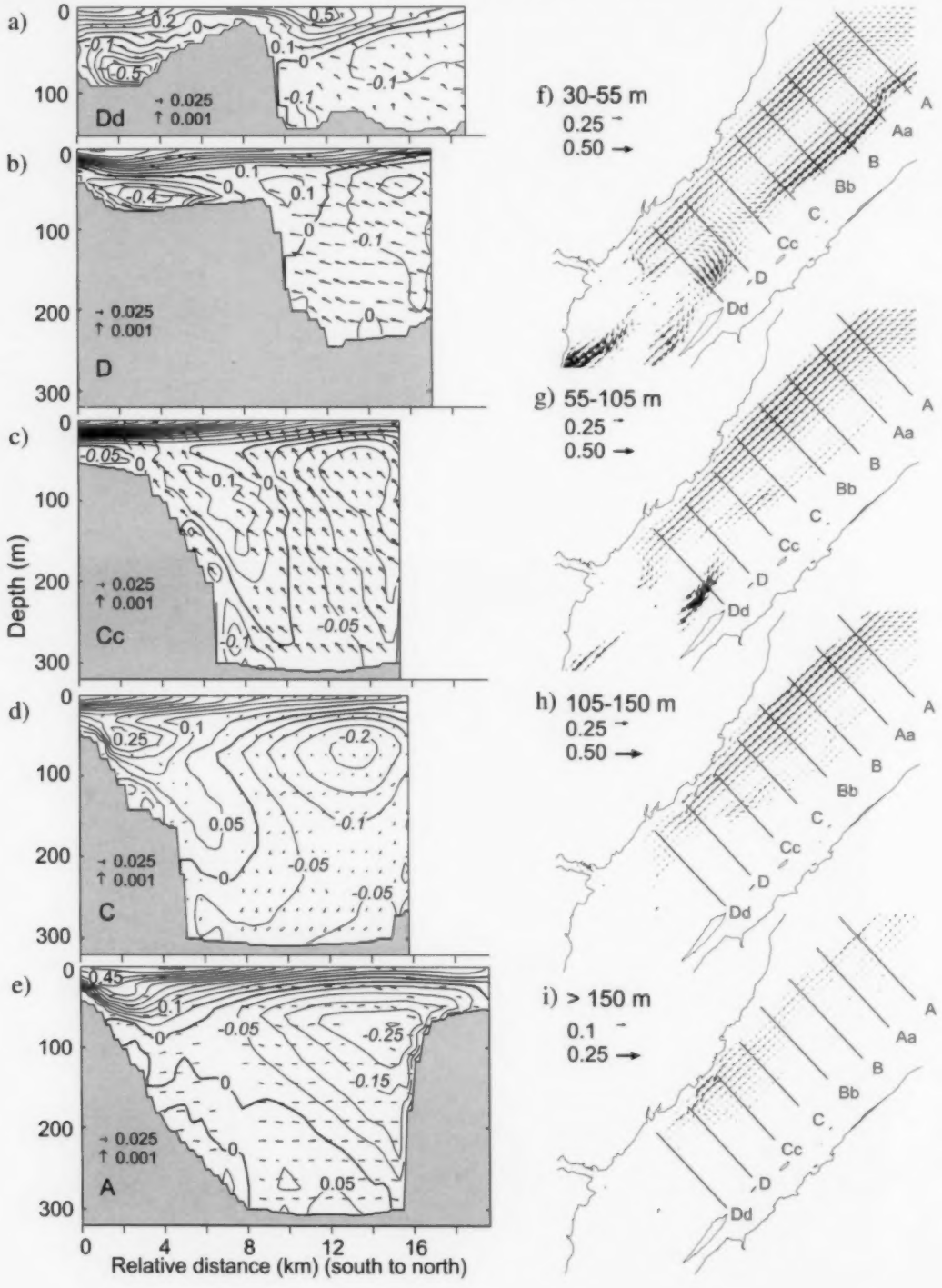
Fig. 3. Mean modeled currents ($m \cdot s^{-1}$) averaged over a fortnightly tidal cycle (14.75 days). (*a–e*) Cross sections corresponding to some transects, the contours representing the *u* component with negative values entering into the page and the arrows representing the *v* and *w* components. (*f–i*) Depth-averaged horizontal currents for the layers indicated. Note that the current scales are different for each layer.



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daily and to their partial mixing with fresher surface water (Therriault and Lacroix 1976; Reid 1977; Saucier and Chassé 2000). This region was shown to be the principal region of CIL erosion in the St. Lawrence Estuary (Saucier and Chassé 2000). *Meganyctiphanes norvegica* is mainly found in the deep oceanic layer in daytime, while *Thysanoessa raschi* occupies the CIL (Berkes 1976; Simard et al. 1986*b*). Both species undergo typical DVM to the upper water column at night, but the duration of their stay in the upper layer to gather food appears to be short (Simard et al. 1986*a*). Therefore, depending on the season (e.g., runoff strength, CIL properties) and horizontal location of the organisms, the relative importance of surface outflow and deeper upstream flow in the transport of the vertically migrating adult euphausiids may vary greatly. Also, the continually changing phase offset between the 12.42-h semidiurnal tidal cycle and the DVM of the euphausiids may lead to complex dispersion and aggregation patterns.

The dynamics of the krill aggregation and dispersion mechanisms in relation to the circulation patterns and the topography at the LC head have not been investigated up to now. Zakardjian et al. (1999) demonstrated general modes of copepod dispersion in an idealized two-dimensional model of the circulation. In the present paper, acoustic observations and the results of a high-resolution model of the three-dimensional tidal circulation are examined in turn and then compared. The three-dimensional circulation combined with the known phototactic response of krill to a barrier isolume (see Simard et al. 1986*b* and references therein; Simard and Mackas 1989; Mackas et al. 1997) is used to explain the formation and dispersion of the rich krill patches. The next section presents the acoustic sampling and numerical model characteristics. The three-dimensional tidal circulation and krill distribution are then described and compared for the five zones of aggregation identified in this paper, each having different circulation dynamics. It is followed by a discussion on the major variability factors affecting the abundance and distribution pattern of krill.

Material and methods

Sampling

Sampling was carried out on four missions in the summers of 1994 (ends of June, July, and August) and 1995 (mid-July) on a regular grid of 10 transects spaced by 7.4 km, perpendicularly to the LC axis (Fig. 1). The grid was surveyed twice during each mission with 3–10 days between each visit. It was visited during daytime only, when krill scattering layers (SL) were at the daytime depth of their DVM and well separated from fish echoes. To keep the survey time of the whole grid within 2 days, a few transects were omitted during some visits. Water properties along the transects were determined with a salinity and temperature profiler at a grid of stations (Fig. 1) (Lavoie et al. 1996). At these stations, zooplankton was also sampled over the whole water column from either a 0.28-m^2 Bongo net or a 1-m^2 Bioness (0.33-mm mesh).

The acoustic system was a Biosonics 102 echosounder equipped with two transducers operating at 120 and 38 kHz. The ping rate was 1·s^{-1} for each frequency, triggered alternately. The fish echoes were separated from the krill SL using the difference in backscattered energy at the two frequencies (Simard and Lavoie 1999). Echointegration along transects was done over a range of 250 m, which included all the krill SL. Only the echoes exceeding the noise threshold profiles for both frequencies were integrated. Distribution maps of krill biomass over the study area were computed from punctual kriging on a grid of 1 km^2 delimited by the survey area (Fig. 1). To take into account the along-channel organization of the krill biomass, the variograms were computed in the cross-channel direction (320°N) and along the main axis of the estuary (50°N). An elliptic neighbourhood of 2 × 16 km oriented along the axis of the estuary was used. For more details on the methods, see Simard and Lavoie (1999).

Three-dimensional numerical modelling

In order to reproduce the three-dimensional circulation in the St. Lawrence Estuary, we make use of a numerical model that was first implemented to produce the St. Lawrence tidal current atlas (Canadian Hydrographic Service 1997; Saucier et al. 1999). This model reproduces the circulation and the density changes associated with tidal propagation and river runoff over hourly to monthly time scales. The model domain extends from Rimouski to the upper limits of tidal influence near Trois-Rivières (~380 km upstream of Rimouski) and at the head of the Saguenay fjord, with 400-m resolution in the horizontal and 20 layers in the vertical. The model is forced from tidal water level constituents at the open boundary and 20 rivers with the most significant runoff. The initial density condition is specified from observed profiles acquired in 1975 in the Upper Estuary and the mean summer condition derived in Forrester (1974) for the Lower Estuary. The tidal circulation that is produced by the model has been validated through comparisons with various current measurements, both time series at depth, and surface drifters deployed at various stages of the tide.

The interaction of the tide with the topography leads to complex three-dimensional currents, sill processes, fronts, internal waves, and other density changes. Some of these features, as they relate to the physics over the sills and the results of the present circulation model, have been examined by Saucier and Chassé (2000). Herein, we extend this analysis to the general tidal circulation in the Lower Estuary. Hourly currents and density changes for a typical semidiurnal cycle (M_2) of mean tidal range as well as residual currents over a fortnightly tidal cycle (MS_f, 14.75 days) were used for the present paper.

Results

Tidal circulation

The configuration of the LC head imprints a strong three-dimensional character to the local circulation and thus to the euphausiid distribution. The main bathymetric features are width reduction, slope steepening, bottom shoaling, and sharp ending with shallow sills (see Fig. 2*b*). In the downstream area (between transects A and Bb, Fig. 1), the channel cross section is relatively constant above 200 m, but the southern slope is less steep than the northern one at greater depths (Figs. 2*b* and 3*e*). The mean circulation shows upstream transport at CIL depths across the full breadth of the channel and at all depths along the north side and downstream transport at the surface, along the edge of the southern plateau, and at depth (Figs. 3*e*–3*i*). Indeed, the ebb to flood transition first occurs along the north shore, at CIL depths (55–105 m), during the hour following low water (LW) (Figs. 4 and 5). It then spreads out over the whole water column (below 30 m) except along the edge of the southern plateau due to the downstream propagation of the density current (Fig. 4).

In the middle area (between transects Bb and Cc), there is a strong constriction of the channel and the slopes become very steep, particularly the northern one (Figs. 1 and 2*b*).

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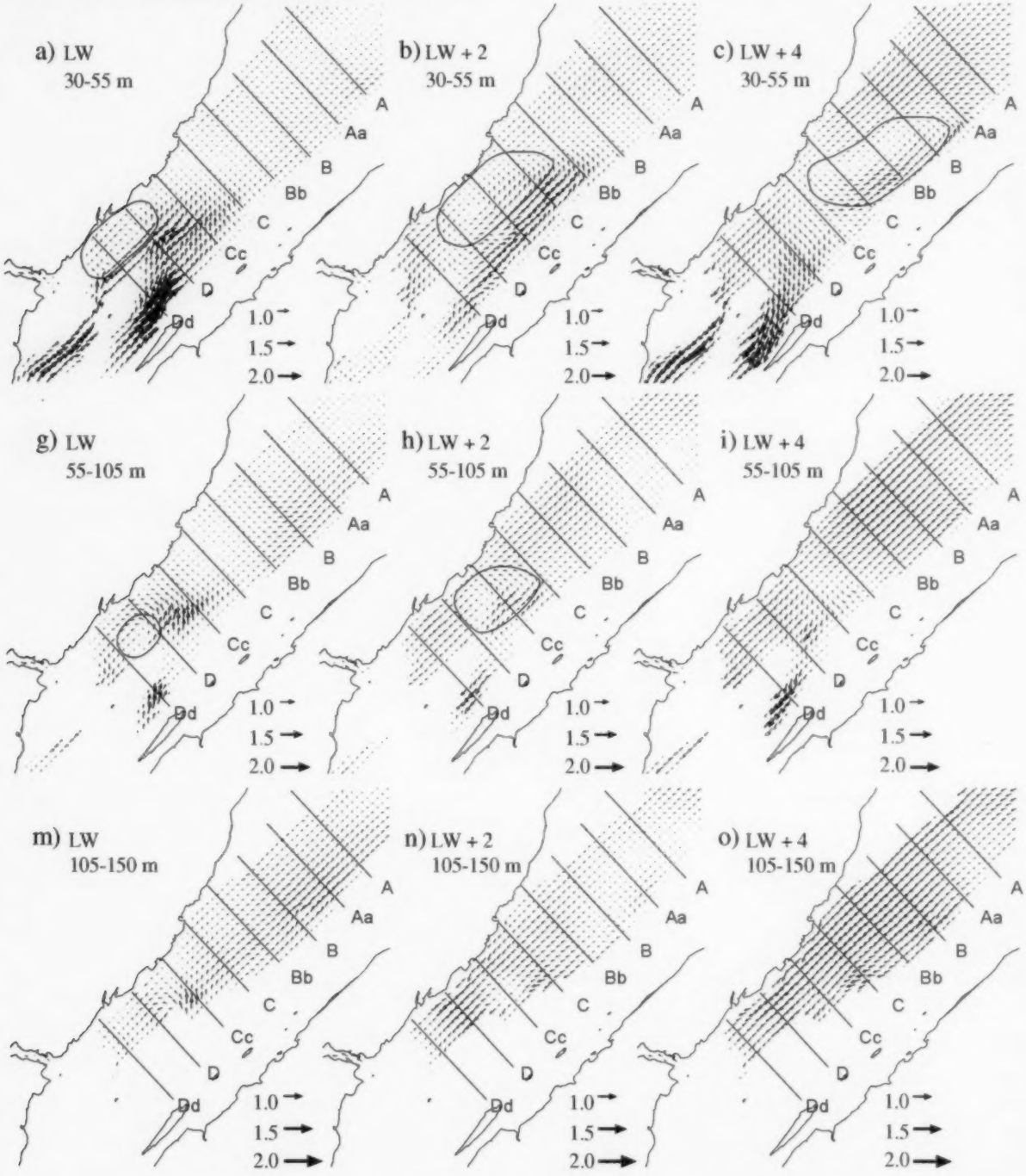
Fig. 4. Hourly horizontal currents ($m \cdot s^{-1}$) modelled over a mean summer semidiurnal cycle (tidal range of 3 m) for the hours indicated after LW and HW at Rimouski. The currents are depth-averaged between (*a–f*) 30 and 55 m, (*g–l*) 55 and 105 m, and (*m–r*) 105 and 150 m. Note that the current scales are different for each layer.



The width of the channel at Cc (below 100 m) is about half that at Bb. This configuration favours strong vertical currents (up to 1 $cm \cdot s^{-1}$) along the steep slopes on both sides of the channel (Figs. 2*b*, 6, 7, 8*c*, and 8*d*). The diversion of the inflowing water towards the SC generates cross-channel currents in the middle area from 2 h before to 2 h after high

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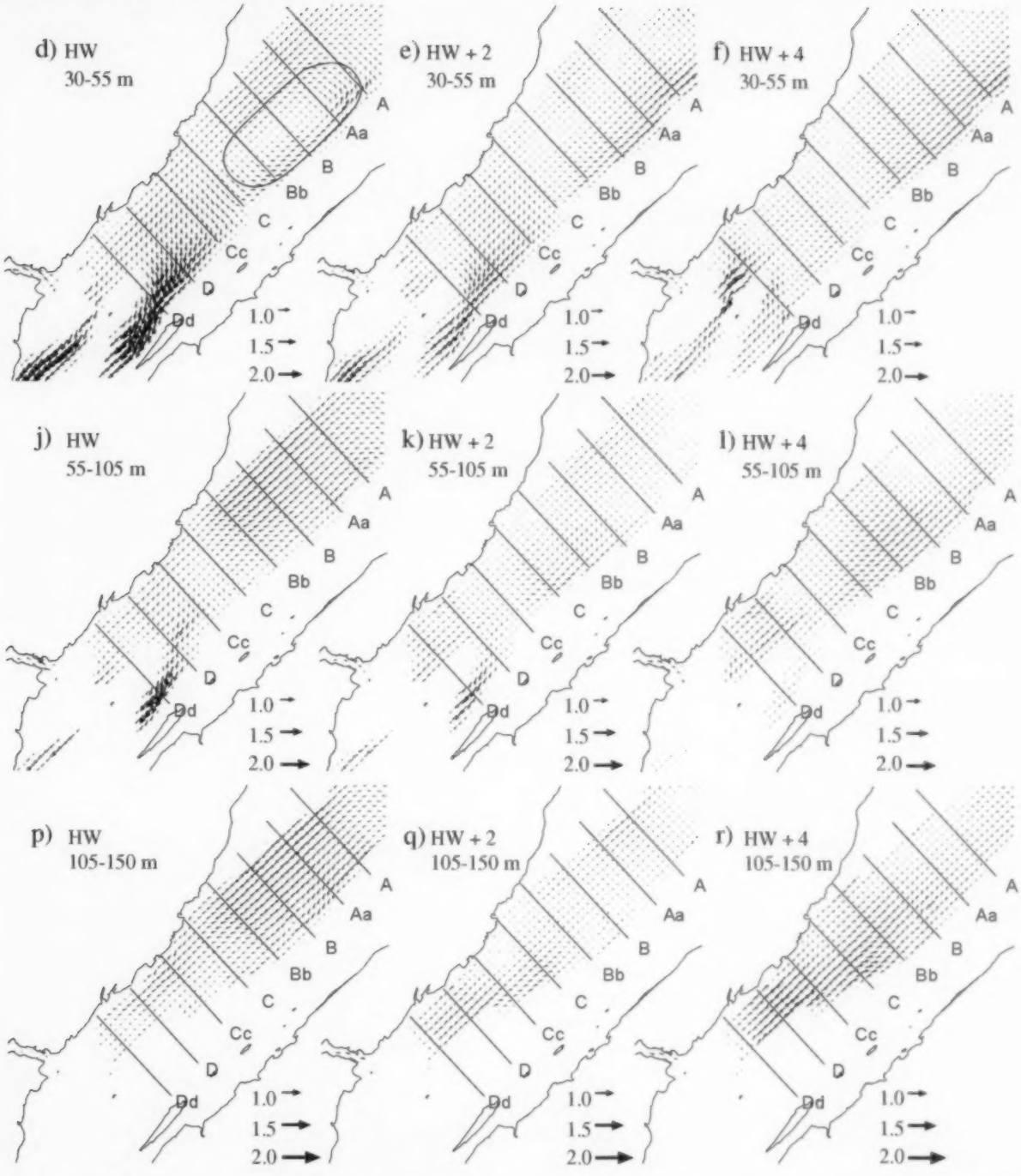
Fig. 4 (*concluded*).



water (HW) (Figs. 2*b* and 4). The mean vertical currents, upward at transect Cc and downward at transect C, suggest the presence of a vertical gyre over the 300-m trough (Figs. 3*c* and 3*d*).

The shoaling area, upstream of transect Cc, is the site of intense vertical motions of deep water (Fig. 7). During the 2 h preceding HW, the upstream speed below 50 m peaks (Figs. 4*i* and 4*o*) and upwelling of CIL water over the sills is maximal. The upwelling stops 2 h after HW (Fig. 7*i*). Strong downwelling follows as the current turns to ebb and as the

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Fig. 5. Hourly velocities and densities (σ_t) during a semidiurnal cycle (same as in Fig. 4) along transect A. Velocities as in Fig. 3.

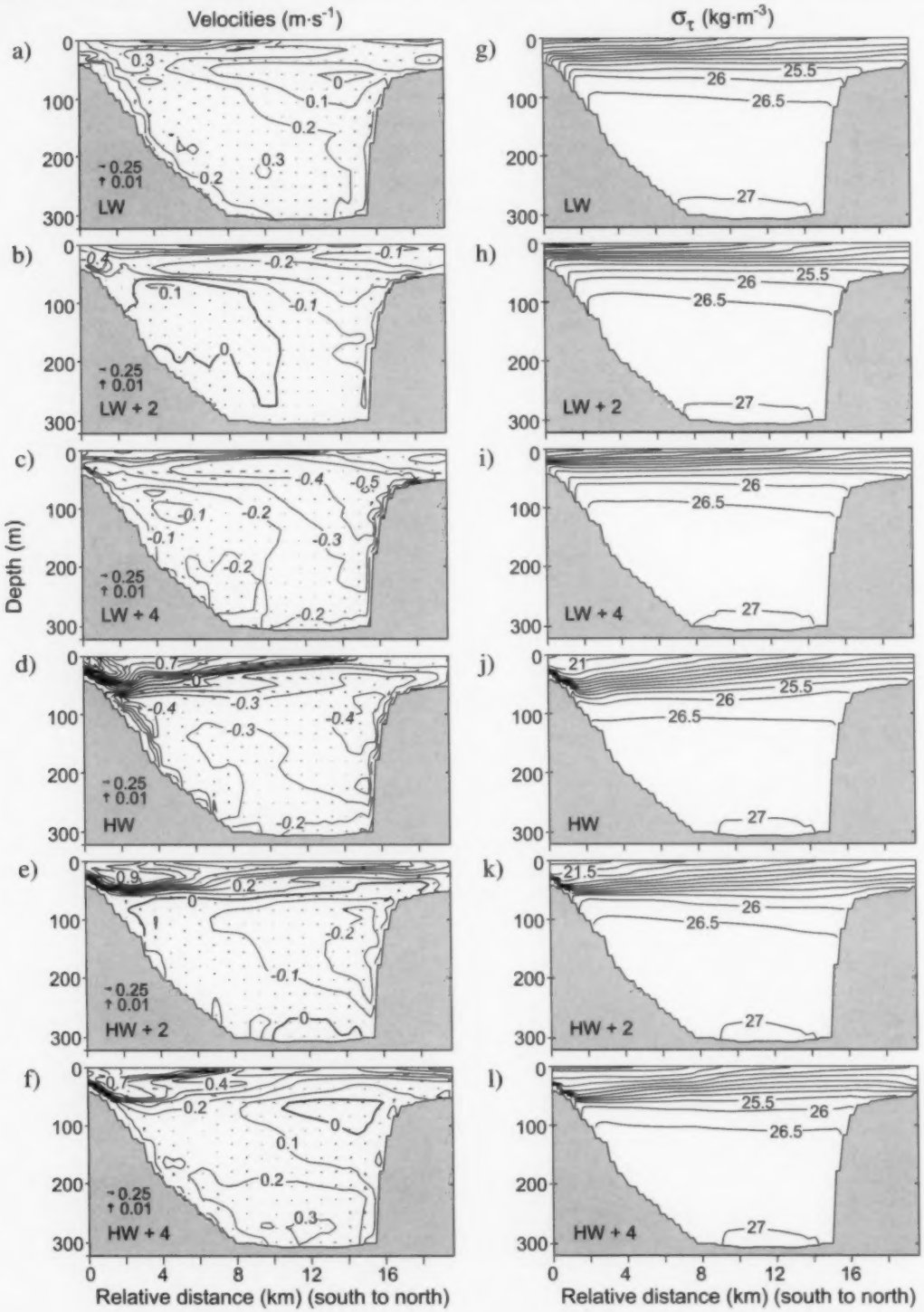

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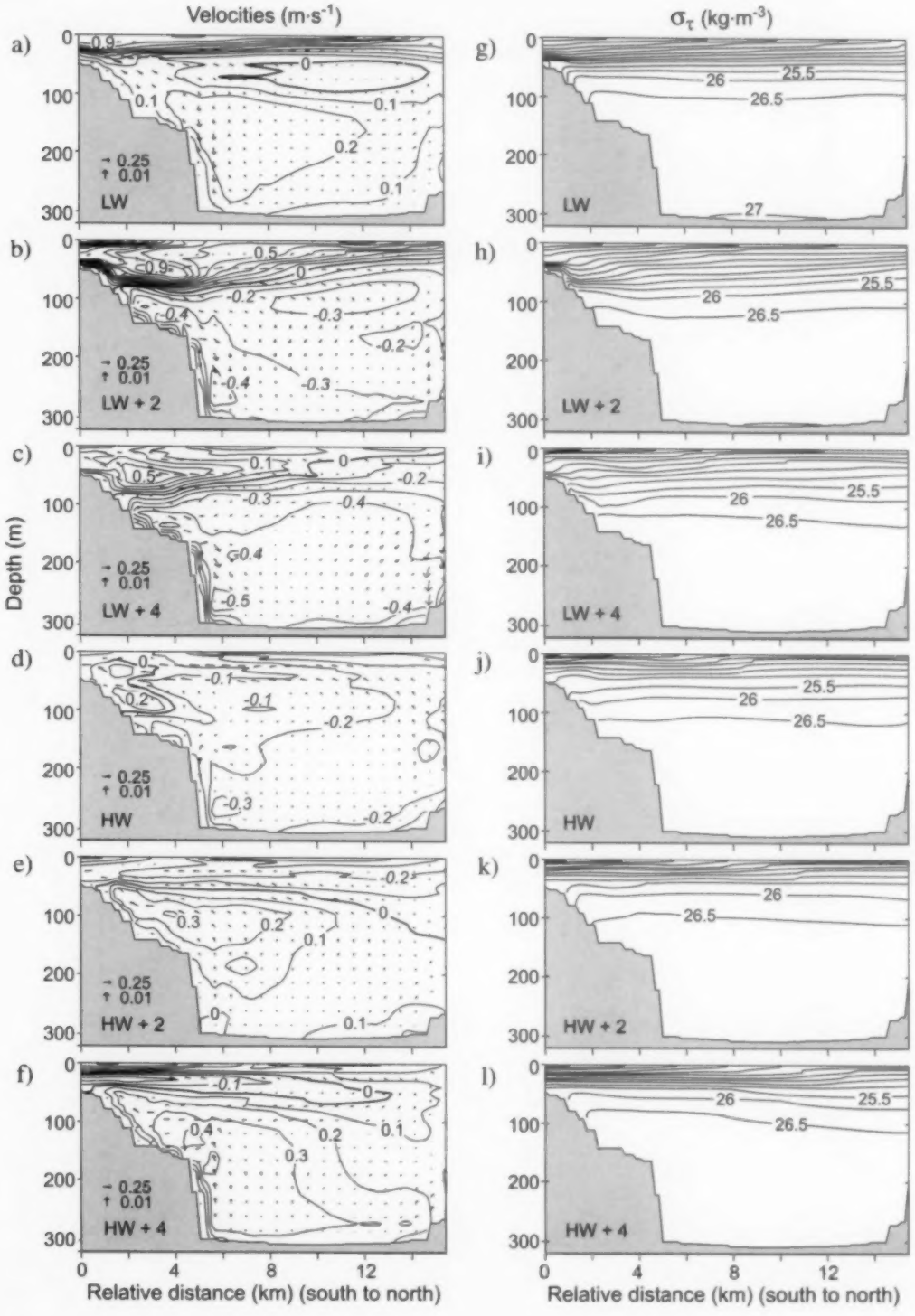
Fig. 6. Hourly velocities and densities (σ_t) during a semidiurnal cycle (same as in Figs. 4 and 5) along transect C. Velocities as in Fig. 3.



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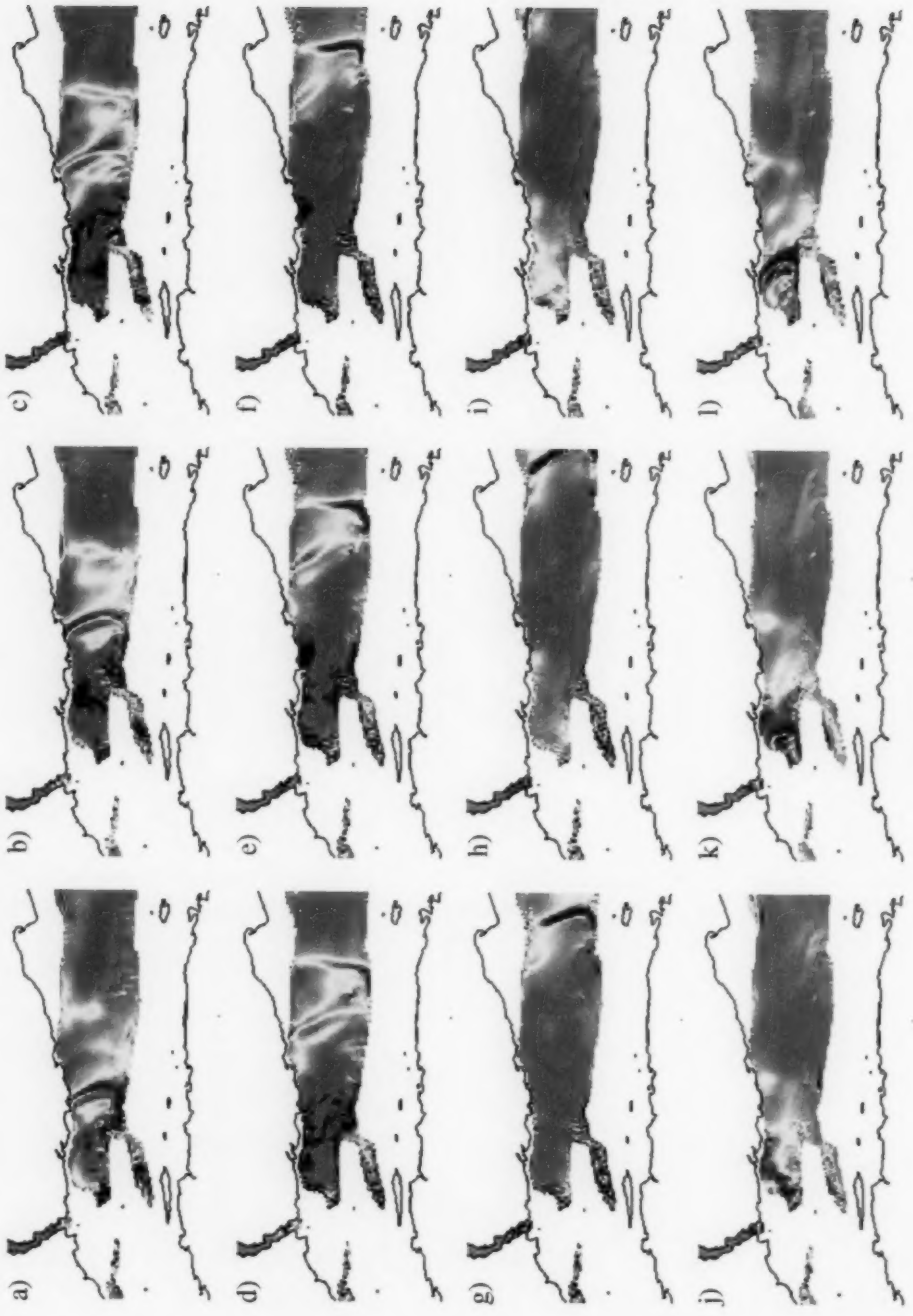

Fig. 7. Hourly vertical velocities (w) depth-averaged between 65 and 75 m over the semi-diurnal cycle: (*a*) LW, (*b*) LW + 1, (*c*) LW + 2, (*d*) LW + 3, (*e*) LW + 4, (*f*) LW + 5, (*g*) HW, (*h*) HW + 1, (*i*) HW + 2, (*j*) HW + 3, (*k*) HW + 4, and (*l*) HW + 5.

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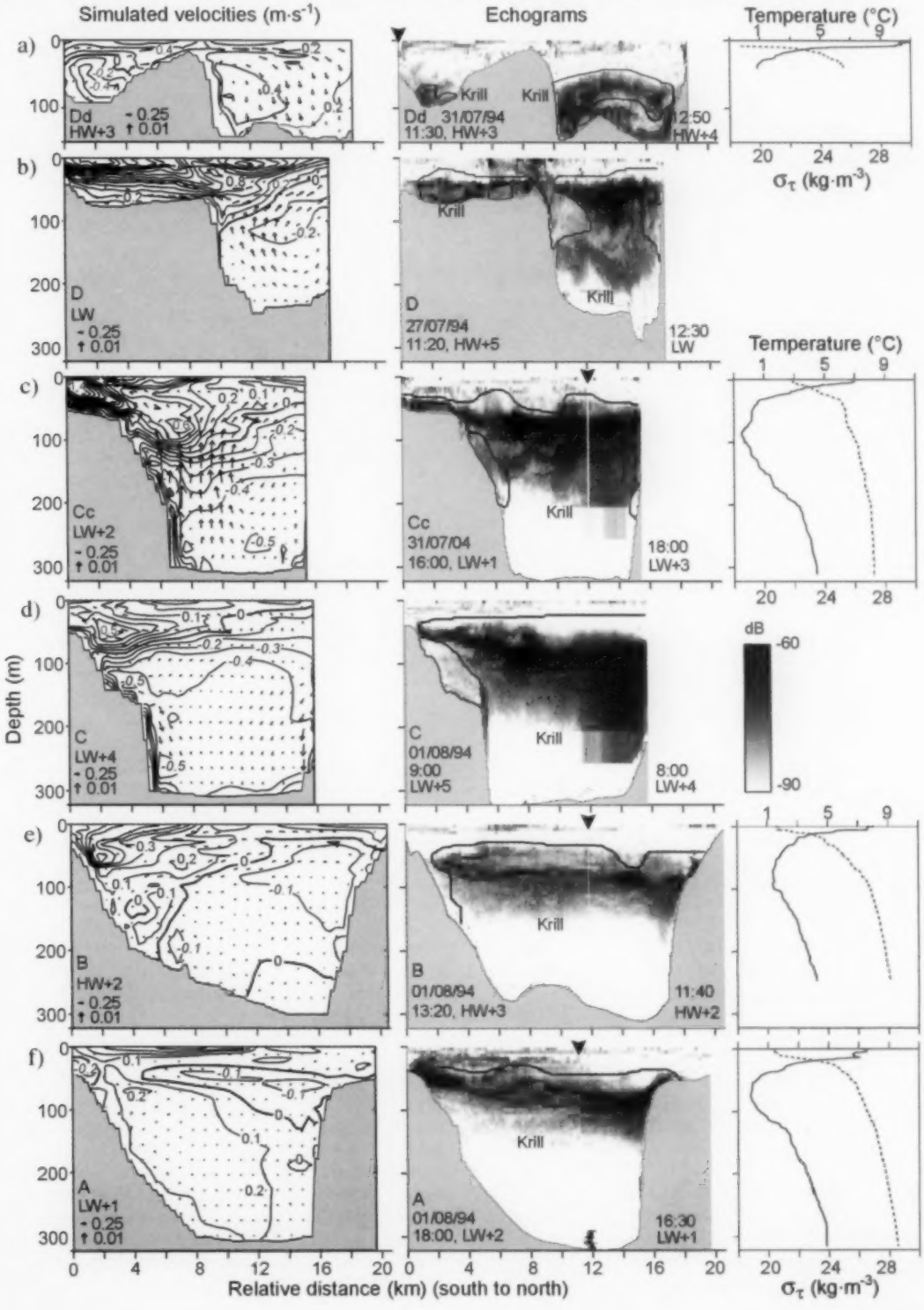
Fig. 8. Typical echointegrated data for different cross-channel sections of the krill SL with corresponding velocities (as in Fig. 3) obtained from the three-dimensional numerical model. The dark lines on the echograms separate krill from fish echoes. Arrowheads on top of the echograms indicate the location of the temperature and density (σ_t, dashed line) profiles, shown on the right.



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previously upwelled dense water sinks back to depth. An internal bore is generated at sill Sb (Fig. 7*k*) and propagates downstream. The maximum downwelling on the downstream side of sill Sa occurs right after the passage of the propagating internal bore (Fig. 7*a*). This downwelling generates a cyclonic gyre at intermediate depths in the LC head area, which subsequently propagates downstream along the edge of the southern plateau (Figs. 4*a*–4*d*). Using a two-dimensional vertically averaged numerical model, Wheless and Klinck (1995) observed that a dense coastal buoyancy flux along a sloping shelf can generate a cross-isobath flow and initiate an offshore cyclonic gyre. The latter is subsequently advected in the direction of propagation of a right-bounded wave. Their results are for a well-mixed sloping shelf, but they can apply here because the process occurs under the pycnocline where the vertical density gradient is weak.

Spatiotemporal distribution of euphausiids

The SL was generally thick (100–200 m) with a denser sublayer in the upper part (e.g., Fig. 8). The mean depth of the densest sublayer varied between each mission but was always found in the CIL. It deepened by ~10 m each month in 1994, going from ~60 m at the end of June to ~80 m at the end of August 1994. In August 1995, the SL was thinner and deeper (densest sublayer depth ~100 m). Both species were represented only by individuals of the oldest 2+ cohort (see Simard and Lavoie 1999), but *T. raschi* was the dominant species (94, 72, and 91% for missions 1, 3, and 4, respectively) except for the second mission where *M. norvegica* was as abundant (52% *T. raschi*). The biomass estimates for the whole study area varied by a factor of 10 over the eight surveys. The highest biomasses were found at the end of June and July 1994, while the lowest biomass was found at the end of August 1994 (details in Simard and Lavoie 1999).

In the downstream area, the SL generally ranged between depths of 30 and 160 m with a 10- to 20-m-thick sublayer of higher density (e.g., Figs. 8*e* and 8*f*). The SL was relatively continuous across the channel with denser patches on one or both sides of the channel. In the middle area, the SL highest densities were found at depths similar to those found downstream (Fig. 8*d*). However, the SL was generally denser and its lower limit was deeper, especially along the northern slope where it extended down to 250 m in the second mission. The SL sometimes extended to the flat and shallow shelves on the northern part of transects A and Aa (Figs. 8*f*, 9*a*, and 9*b*) and on the southern part of transect Cc (Figs. 8*c*, 9*c*, and 9*d*), where they often came into contact with the bottom. In the shoaling area, the vertical distribution of the SL, when present, was modulated by the M_2 tidal cycle and was thus very variable (Figs. 8*a* and 8*b*).

Figure 9 shows the horizontal distribution of the vertically integrated krill biomass for the eight surveys from which five discrete zones of aggregation may be identified (1 to 5 in Fig. 2*a*). An aggregation was present along the north slope (1) of the LC on every visit of the study area (Fig. 9). It formed an elongated band along the 100-m isobath, from the upper corner of the study area up to Les Escoumins. Patchiness was observed within the band. A large and dense aggregation was generally present off Les Escoumins (2), over the >300-m trough, with the densest part close to the north shore. It sometimes extended across the full breadth of the channel and along the southern slope (5), giving a U-shape to the vertically integrated biomass (Figs. 9*a*, 9*f*, and 9*h*). Euphausiids were also found in significant densities in the shoaling zone (4) on half of our surveys (Figs. 9*b*, 9*c*, 9*d*, and 9*g*). On these occasions, the SC (3) also contained more euphausiids. The krill distribution pattern in the study area changed with the fortnightly tidal cycle. Before neap tide (N) (about spring tide (S) + 5 days) (Figs. 9*a*, 9*f*, and 9*h*), the distribution was U-shaped with a greater abundance near Les Escoumins and with few krill in the head area. Just after neap tide (about N to N + 3 days) (Figs. 9*b*, 9*d*, and 9*g*), more krill were found in the SC and in the shoaling zone and the U-shape had disappeared. During spring tide (Fig. 9*c*), the distribution was more elongated, with krill found along the north slope over the whole study area, and the abundance of krill in the SC zone was maximal. The distribution on 24–25 August 1994 when krill were not abundant and dispersed (Fig. 9*e*), did not show any strong pattern. In the following section, we analyse the role of the tidal circulation as a governing process in the distribution of the biomass in the five zones identified in Fig. 2*a*.

Euphausiid distribution and circulation in the five zones

To help the reader, the principal circulation characteristics responsible for the observed distribution are sketched in Fig. 2*b*.

North slope band

The northeast corner of the study area is the entrance corridor of the krill in the study area. The euphausiids found in this zone are mainly advected towards Les Escoumins by the along-channel flow (Figs. 2*b*, 3*e*–3*i*, and 5). At CIL depths, the latter is inflowing from LW + 1 h to HW + 2 h (7 h) and outflowing from HW + 4 h to LW (3 h) (Figs. 4*g*–4*l*, 5, and 8*f*). They are also advected towards and over the shelf by the tidally forced upwelling currents present during the few hours preceding HW (Figs. 5*c* and 7*d*–7*f*). Because of the tendency of krill to stay below a barrier isolume, the krill involved in the upwelling circulation concentrate along the LC northern slope, leading to the observed SL abutting the plateau's edge (Fig. 8*f*).

Les Escoumins' patch

Two physical processes contribute to the high vertically integrated biomass observed in this zone on Fig. 9: (*i*) the general slowing down and diversion of the inflow towards the SC (Fig. 4) and (*ii*) the increased vertical motion, both positive and negative (see Figs. 3*c*, 3*d*, 6, and 7). The first one results from the rapid bottom shoaling and deepwater blocking occurring at the head of the LC and from the relatively deeper sill bounding the SC (see Fig. 2*b*). The second one is favoured by the steepening of the slopes (see Fig. 2*b*) and contributes to the accumulation and vertical spreading of the inflowing krill (Figs. 8*c* and 8*d*). Downwelling occurs over the whole water column during the passage of the seaward-propagating internal bore from LW at the upstream end of the zone to 2 h after LW at its downstream end (Figs. 7*a*–7*c*). The cyclonic gyre found right behind also generates strong vertical motion through the lateral transport of the intermediate density water formed in the SC (see next section)

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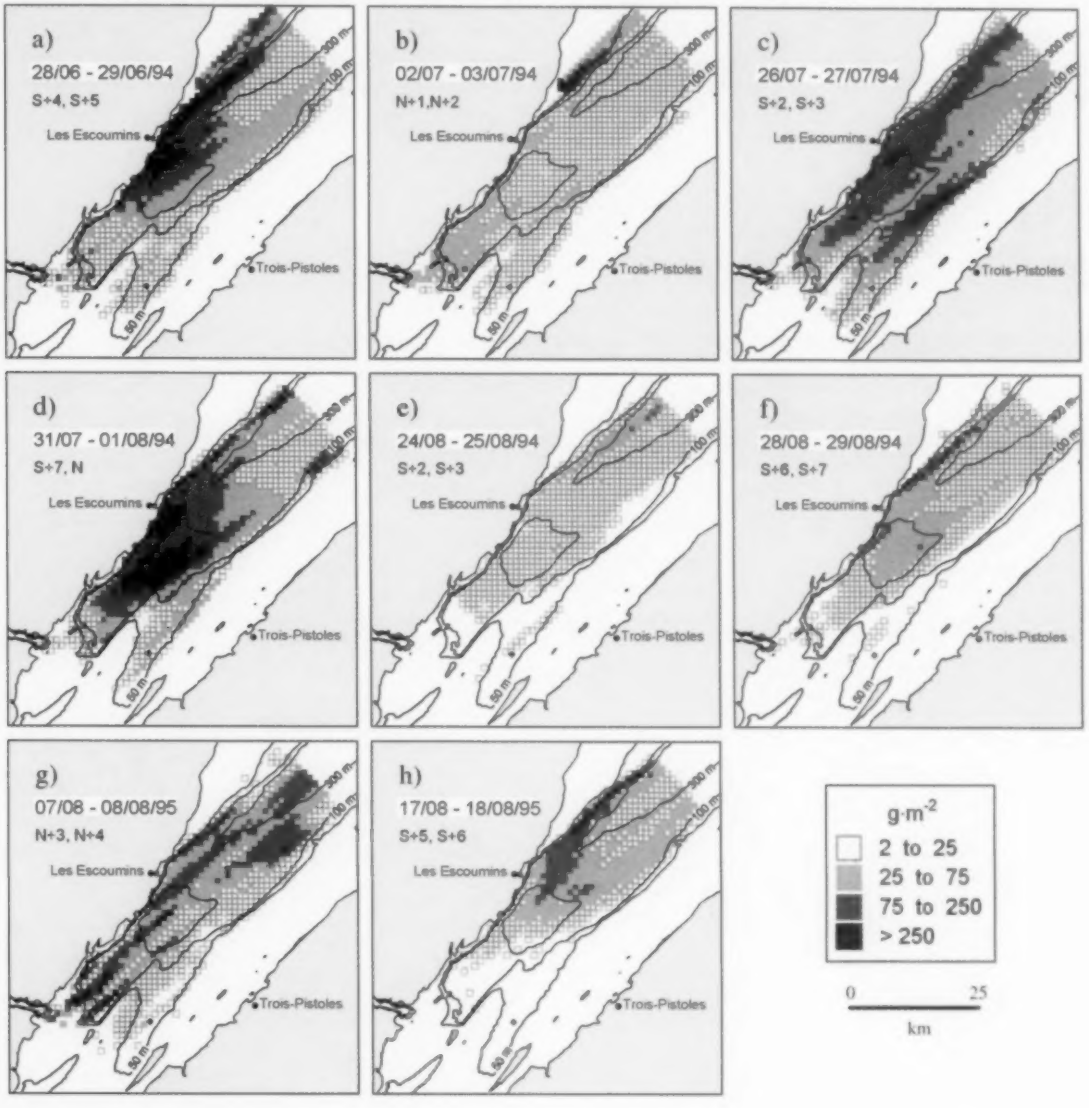
Fig. 9. Horizontal distribution of the vertically integrated biomass of krill for the eight visits to the study area. Kriged grid mesh: 1 × 1 km. The fortnightly tidal phase (number of days after spring (S) or neap tide (N)) is indicated for each visit.



(Figs. 4*g*, 4*h*, 6*a*, and 6*b*). A vertical divergence is observed around 60 m with upwelling above and strong downwelling below, which lasts for ~3 h. The downstream propagation of the density current along the southern slope of the LC constrains the flood stream, and thus krill inflow, to the north side of the channel (Figs. 4*b*, 4*c*, and 4*h*). Strong upwelling expands to the whole water column after the passage of the gyre (Figs. 6*d* and 7*e*–7*h*), with maximum intensity occurring in the upstream part (around transect Cc) 4 h after LW (Fig. 7*e*). The strong downwelling (e.g., Fig. 8*d*), which is important enough to characterize the mean circulation at transect C (Fig. 3*d*), favours the downward entrainment of the euphausiids and would explain the general downward stretching of the SL (Figs. 8*c* and 8*d*), while the upwelling would lead to the concentration of krill at their barrier isolume.

South Channel

The SC is not a site where large amounts of krill aggregate (Fig. 9), but it plays a major role in the hydrodynamics and hence on the krill distribution. The deeper sill Sa (70 m), compared with sills Sb and Sc (<40 m), permits the inflow of the top part of the CIL in the SC. Moreover, water below the sill crest can be advected over it under the influence of the barotropic tide. Maximum upward particle displacement, h, which is constrained by density stratification, is approximately equal to $\pi\bar{u}N^{-1}$ (see Farmer and Denton 1985) where $\bar{u}$ is the maximum sectionally averaged velocity

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and N is the Brunt–Väisälä frequency. Mean values of $\bar{u}$ = 0.2 m·s^{-1} and N = 0.01·s^{-1} for the depth range 40–150 m yield a blocking depth (h) of ~60 m (see Saucier and Chassé 2000), which is in the range of the values calculated by Reid (1977). Thus, particles deeper than h below the sill crest will tend to be "blocked" by the sill, i.e., prevented from flowing over it. More CIL water (and krill) will thus be forced over sill Sa (down to depths of ~130 m) than over sills Sb and Sc (down to depths of ~80 to 100 m).

Strong upwelling of CIL water occurring during the 3 h preceding HW (Figs. 4*i* and 7*d*–7*f*) contributes to the aggregation of krill along the LC southern slope. The krill advected in the SC aggregate close to the bottom in the deepest parts, as shown in Figs. 8*a* and 8*b*. The upwelling stops 3 h after HW (Figs. 4*k*, 4*l*, and 7*j*), and the shear between the outflowing surface currents and the upwelled CIL water leads to strong mixing (Saucier and Chassé 2000), which generates a new water mass of intermediate density. The downstream propagation of this water mass results in a strong gravity current on the outer slope of sill Sa and along the south slope of the LC (Figs. 4*a*, 4*b*, 4*g*, 4*h*, 4*m*, 7*a*, and 7*b*). The dense plume is advected offshore, i.e., towards the north shore, and is recirculated towards the LC head forming the cyclonic gyre at intermediate depths (Figs. 4*a* and 4*g*). A large part of the krill is flushed out of the SC with the relatively dense plume and the balance is trapped in its deepest part. Krill present along the south slope would be advected downstream by the strong outflowing density current (Fig. 6*b*), which is clearly visible in the mean currents of Figs. 3*d*, 3*f*, and 3*g*.

Shoaling zone

On average, the krill abundance at the head of the LC is much lower and more variable than downstream due to the high variability of the flow in this zone. The krill is advected upstream along the north shore mainly when upwelling is occurring over sills Sb and Sc (maximal during the 2 h preceding HW) (see Figs. 4*c* and 4*i*). A large part of the flow is channelled towards sill Sb during flooding. The barotropic elevation of the deep water masses at the head of LC brings the SL closer to the surface, but upwelling is stronger along the sides of the channel and at the sills (Figs. 7*c*–7*f*). The krill swimming down to maintain their light level will aggregate at the slopes and will gradually find themselves in denser waters. Intense downwelling occurs after current slack when the dense upwelled waters return back towards their initial depths (Figs. 7*i* and 7*j*), advecting krill downstream and at depths greater than the source SL, as shown in Fig. 8*a*. The internal bore propagates downstream soon after (Fig. 7) and generates transient variations of SL depths, contributing to its vertical spreading. The cyclonic gyre generated in the shoaling zone after the passage of the internal bore would advect krill present in the SC (if any) in the shoaling zone. As the gyre propagates downstream, the upstream advection of krill from the Les Escoumins patch resumes (Fig. 4).

South slope band

This zone is the exit corridor of the study area for the krill (Figs. 3 and 4). The krill found along the south slope that are not recirculated towards the north shore by the transverse currents found at the leading edge of the propagating cyclonic gyre are mainly flushed out of the study area by the ebb currents. These latter are stronger along the slope and present from 2 h after HW to 1 h after LW (Figs. 4 and 5). They result in strong outflowing residual currents above 100 m (Figs. 3*d*–3*g*). Currents towards the south shore are also observed just behind the propagating gyre (Figs. 4*c*, 4*d*, and 4*i*). They would advect some krill from the rich Les Escoumins patch towards the outflowing south slope current.

Discussion

The head of the LC is the site of a quasi-permanent rich krill aggregation during summer (Simard et al. 1986*b*; Simard and Lavoie 1999) because several oceanographic processes concur there to "import" and then "concentrate" and "accumulate" adult krill in this area of the St. Lawrence. The rapid fluctuations of the total biomass of this aggregation (Simard and Lavoie 1999) indicate, however, that the "retention" in the LC head (<75 km) is deficient on time scales larger than a week. The results clearly show that the complex aggregation and dispersion processes draw from a broad range of scales, from the minute-scale reaction of krill to in situ light up to the large-scale estuarine circulation, including the important semidiurnal and fortnightly tidal cycles. High-resolution biomass data provided by high-frequency acoustics, proper statistical mapping methods, and high-resolution synoptic current fields obtained from a validated three-dimensional numerical model proved here to be a minimum requirement to cope with the great complexity of this area of the St. Lawrence Estuary. We distinguish below some important components that are responsible for the aggregation, patchiness, and fluctuations of krill in the Saguenay – St. Lawrence Marine Park.

Ontogeny of vertical migration and estuarine circulation

The preponderance of the second cohort of both euphausiid species at the LC head was attributed by Simard and Lavoie (1999) to the horizontal sorting of the various life stages by the two-layer circulation system, since they occupy different levels in the water column. The same mechanism has been invoked to explain the horizontal distribution of krill and size zonation in other systems (e.g., Mackas et al. 1997; Ichii et al. 1998). The strong surface outflow appears to continually flush the eggs and nauplii, which are found in the surface layer, towards the southern Gulf of St. Lawrence (Berkes 1976; Y. Simard et al., unpublished data). With the development of DVM in the next stages, gradually deeper daytime levels are occupied by older stages and less time is spent in the surface layer during DVM (Lacroix 1961), possibly just for collecting food subsequently processed at depth (Simard et al. 1986*a*). The distribution of the old stages in the upstream-moving deep layers, for most of the time, results in their net transport towards the head of the LC. Besides, the different vertical distribution of the two euphausiids species relative to the depth of peak inflow could explain why *M. norvegica* is less abundant than *T. raschi* in the study area. *Meganyctiphanes norvegica* is found in the oceanic layer where the upstream residual current is much lower (2 cm·s^{-1}; Ingram 1979) than in the CIL

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(10–15 cm·s^{-1}; Ingram 1979) where *T. raschi* is found. A copepod vertical migration model coupled to a simplified two-dimensional circulation model of the Lower St. Lawrence Estuary using mean long-term current values (Zakardjian et al. 1999) illustrates this process. But how precisely this dispersal and import loop works, notably with the tidal and seasonal circulation pulses, requires further research and detailed simulation. Some of the key factors are examined in the sections below.

Light barrier and vertical motion

There is much evidences that in situ light levels exclude vertically migrating organisms from the upper water column during daytime (e.g., Simard et al. 1986*a*; Gal et al. 1999). The avoided light level varies among species, developmental stages, and individuals in a population. It determines a barrier isolume below which the organisms are found in the water column and that controls the ascent and the descent of the SL during DVM (e.g., Simard et al. 1986*a*), when additional altering biological (e.g., predators, food) or chemical factors (e.g., temperature, oxygen, salinity) do not come into play. This negative phototactism appears to be a key factor in concentrating krill in upwelling systems, the latter swimming downward to stay in the safe dark zone when advected up (e.g., Simard et al. 1986*b*; Mackas et al. 1997). This mechanism would explain the stronger aggregations found along the slopes (upwelling) and the downward stretching of the SL in the Les Escoumins zone (downwelling). This is indeed where the highest biomass is found. It results from the convergence of the horizontal currents, which provide a constant inflow of krill, but in large part from the strong and long-lasting vertical currents, both upward and downward. The maximal modelled vertical velocities are of the order of 1 cm·s^{-1}. This corresponds to a swimming speed of <1 body length·s^{-1} for the euphausiids reacting to the light barrier, which should be sustainable for some hours (Hardy and Bainbridge 1954). However, the krill moved down to larger depths would not react as rapidly as in the case of positive vertical motion because their survival may not be as threatened as it would be by increasing light levels. In the shoaling zone, high velocities are reached as the water moves along the face of sill Sb (in a relatively thin layer) during maximum upwelling. Vertical velocities obtained from an acoustic doppler current profiler at sill Sb reached up to 15 cm·s^{-1}. Krill present in this layer would likely be unable to fight this intense upwelling current and would be flushed into the Upper Estuary, as evidenced by zooplankton sampling done upstream of our study area by Courtois et al. (1982). Some krill would also be advected over sill Sc and into the Saguenay fjord. A particular mechanism, not described before, is acting in the shoaling zone. The krill advected up at sills Sb and Sc during intense upwelling will find themselves in a denser water mass when swimming down to stay at the same light level. As the upwelling stops and the flow turns to ebb, the dense water returns towards its initial level, advecting krill off the sills area and below the main SL. It could be called a "piling up" mechanism. Both effects contribute to increasing the density of krill per square metre in those areas where vertical motions are important.

The predominant southwesterly winds over the region are also favourable to upwelling along the north shore. They contribute to enhance the abundance of krill along the north slope of the LC in the Lower Estuary (Sameoto 1976; Simard et al. 1986*b*). Krill advected in shallow areas, such as the shelves bordering the LC, the SC, or the Upper Estuary, would concentrate near the bottom when swimming down to avoid the more intense light level. They would become an easier prey for their predators (see Kaartvedt et al. 1996), notably baleen whales and both bottom and pelagic fishes, being more visible, unacclimated to the higher light level, and unable to escape. Advection of krill during their DVM to the surface layer would enhance the risk of trapping in shallow areas during their descent, especially when flooding occurs at night. Figures 9*a* and 9*b* suggest that krill advection over the northern plateau was more important at the beginning of the summer of 1994 when the mean depth of the dense SL was shallower. The change in the light extinction coefficient, and hence in the isolume depth, due to variation in phytoplankton concentration or turbidity could explain the gradual deepening of the SL during the summer of 1994 (e.g., Kaartvedt et al. 1996; Mackas et al. 1997). The turbidity is indeed higher during spring freshet, due to land drainage, and gradually decreases thereafter with runoff (Sinclair 1978).

Neap versus spring tide pattern

The modulation of the circulation by the fortnightly tidal cycle changes the distribution and concentration of krill in the study area. Indeed, the processes regulating the CIL mixing at the head, such as freshwater runoff and tidal strength, greatly influence the CIL upstream advection; hence the import of krill. During spring tide, the higher velocities and the reduced stratification, resulting from stronger mixing, both concur to reduce the deepwater blocking at the sills (Farmer and Freeland 1983), as observed by Wang et al. (1991) and Ingram (1983). The volume of CIL water going over sills Sa and Sb does not change in the same proportion due to their different configurations (Farmer and Freeland 1983). During neap tide, the flood stream of dense water going over sill Sb represents only 7% of the one going over sill Sa (Saucier and Chassé 2000). This percentage increases up to 70% from neap to spring tides, while the net stream over both sills triples. Thus, the volume increases by factors of 19 and 1.9 over sills Sb and Sa, respectively. This would lead to an increased and deeper longitudinal transport of krill along the north shore, since the diversion of the currents towards the SC is reduced. The intensified mixing in the SC and Upper Estuary of a larger volume of CIL water would result in a stronger outflow along the south shelf at ebbing tide. The increased advection of krill along both slopes would build the more elongated pattern of krill distribution in Fig. 9*c*. Flushing of krill upstream and downstream of the study area would thus be more intense during spring tide. At neap tide, the stronger deepwater blocking is unfavourable to advection of krill over the sills, and the stronger cyclonic circulation helps to retain the krill in the study area. Moreover, the combination of the circadian DVM with the fortnightly circulation appears to influence the advection of krill up to the study area. For the spring and summer of 1994, the night stay of the euphausiids in the surface layer generally coincided with the flood during spring tides and with the ebb during neap tide. This would, on average, favour the net up-

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stream advection of krill over the fortnightly cycle or, at least, reduce their downstream advection during their night stay in the surface layer.

Freshwater discharge and seasonal cycle

Freshwater discharge is an important factor controlling the strength of the two-layer estuarine circulation (Neu 1970). A seasonal modulation of the CIL advection and hence of the pumping of krill from the Gulf of St. Lawrence and lower reaches of the Estuary up to the LC head is thus expected. During winter, the surface layer and the CIL form a unique layer (Banks 1966). The reduced stratification and the low freshwater discharge do not support strong upstream advection of krill in the deep layer. The krill may then be found mainly in the downstream area of the Lower Estuary and in the Gulf. In spring, the high freshwater discharge rapidly stratifies the water column, isolating from the surface the cold water formed during winter (Banks 1966). The new CIL quickly deepens (Neu 1970) and its advection towards the head is enhanced. However, the maximum upstream advection at CIL depths does not coincide with the spring freshet; rather, it would occur in the period following the reduction in discharge, i.e., in June and July (Tee and Lim 1987). The high deepwater blocking at the head, resulting from the increased stratification, is likely responsible for this delay. The advective field during summer would thus be optimal for the import of krill. The upstream transport is maximal in the CIL where the krill probably reside for longer periods, due to both the extended daytime and the abundance of food in the surface layer (Simard et al. 1986*a*), while the flushing in the surface layer is lower than during spring and fall. Maximal biomass of both species at the head of LC in 1994 was indeed observed in June and July. We may expect that the changes in the current regime in fall (second runoff peak) and the eroded CIL would tend to decrease the krill biomass in the study area by reducing the CIL upstream advection and enhancing the flushing in the upper layer.

The freshwater runoff also seems to influence the local biomass on a shorter time scale. The large drop in biomass (92% in 24 days) between missions 2 and 3 (Simard and Lavoie 1999) coincided with a large reduction in runoff (not shown). Deepwater renewal in a fjord has often been linked to low freshwater runoff due to a lower blocking at the sill (e.g., Allen and Simpson 1998). This process could explain the low volume of CIL water and krill biomass observed on 24–25 August 1994 (Lavoie et al. 1996). The sharp drop in runoff was accompanied by large tidal amplitudes (spring tide) and strong westerly winds, which would all have contributed to an intense upwelling of CIL water over the sills and to a strong mixing with the surface layer. This is evidenced by a satellite image of 25 August 1994 (Lavoie et al. 1996) in which an exceptionally large area of cold surface water was observed far upstream in the Upper Estuary and in the Saguenay fjord. All these factors would have led to an unusually strong flushing of krill out of the study area.

In conclusion, the krill aggregation patterns at the LC head are strongly three-dimensional and controlled by the interaction of the M_2 tidal currents with the negative phototactism of the organisms. We have described five major zones of aggregation, each with its particular circulation dynamics. The very steep channel slopes found in the middle area favour strong vertical currents, which act to concentrate and pile up krill. Combined with the diversion of the inflowing current towards the SC and the krill behaviour, this explains why the krill aggregation of the Saguenay – St. Lawrence Marine Park is centered in the area between Les Escoumins and the Cap de Bon-Désir. The CIL advection rate, to which the abundance of *T. raschi* seems to be closely linked, is controlled by the hydraulic processes occurring at the sills bounding the channel head. The latter being modulated by the fortnightly tidal cycle, we observed changes in the krill distribution with this cycle. Krill retention is stronger close to neap tide, due to the stronger deepwater blocking at the sills, which favours cross-shore currents at SL depths. Close to spring tide, more CIL water is advected over sills Sb and Sc, the circulation is less cyclonic, giving an elongated pattern of krill distribution, and more krill are flushed out of the study area. Other factors affecting the stratification strength, such as runoff, also influence the deepwater blocking at the sills. Krill abundance and distribution are thus largely variable on a time scale going from hours with the semidiurnal tidal cycle to years with the runoff cycle. Interannual changes in climate would also affect these dynamics. The result of this complex aggregation/dispersion mechanism is determining where and when the food would meet the requirements of the predators feeding in the Marine Park, notably baleen whales and pelagic fish.

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Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*)

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Abstract: We describe the use of laboratory trials to compare substrate-specific behaviour and susceptibility to predation of hatchery-reared (HR) versus wild summer flounder (*Paralichthys dentatus*) juveniles. HR fish spent significantly more time swimming in the water column than wild fish and took significantly longer to become cryptic on the benthos than wild fish, regardless of substrate type. In predation trials with a blue crab (*Callinectes sapidus*) predator, naïve HR fish were significantly more susceptible to predation than wild fish. Antipredator-conditioned HR fish were significantly less susceptible to predation than naïve HR fish but significantly more susceptible than wild fish, irrespective of substrate. The modified behavioural patterns and increased susceptibility to predation of HR individuals observed in this study indicate that flounder reared in psychosensory-deprived hatchery environments may be poorly equipped to survive in natural habitats; they also indicate that it may be possible to mitigate detrimental behavioural patterns by exposing naïve HR fish to natural stimuli before release into natural environments. These results have important implications for stock enhancement, suggesting that stocked organisms are more likely to achieve postrelease survival if they are conditioned with natural stimuli prior to release into the wild.

Résumé : Nous décrivons le recours à des essais de laboratoire pour comparer le comportement spécifique du substrat et la vulnérabilité à la prédation chez des cardeaux d'été (*Paralichthys dentatus*) juvéniles, d'élevage et sauvages. Les poissons d'élevage passaient nettement plus de temps que les poissons sauvages à nager dans la colonne d'eau, et prenaient nettement plus de temps pour se dissimuler dans le benthos, quel que soit le substrat. Dans des essais de prédation faisant appel au crabe bleu (*Callinectes sapidus*), les poissons d'élevage novices étaient nettement plus vulnérables à la prédation que les poissons sauvages. Les poissons d'élevage conditionnés à un comportement antiprédateurs étaient nettement moins vulnérables que les poissons d'élevage novices, mais nettement plus vulnérables que les poissons sauvages, quel que soit le substrat. Les changements dans le comportement et la plus grande vulnérabilité à la prédation chez les individus d'élevage observés dans notre étude indiquent que les cardeaux élevés dans le milieu appauvri sur le plan psychosensoriel que représente l'écloserie peuvent être mal armés pour survivre dans un habitat naturel; ils indiquent aussi qu'il est possible d'atténuer les patrons comportementaux nuisibles en exposant les poissons novices à des stimuli naturels avant de les lâcher dans le milieu naturel. Ces résultats ont des incidences importantes pour le rétablissement des stocks, car ils font ressortir que les organismes ensemencés sont plus susceptibles de survivre après le lâcher s'ils ont été conditionnés par des stimuli naturels avant leur libération dans le milieu naturel.

[Traduit par la Rédaction]

Introduction

Commercially important marine fish and invertebrate populations are declining worldwide in response to overexploitation and habitat degradation. One result of these declining fisheries stocks has been an effort to identify and impose effective management methods in impacted areas. For example, overexploited stocks may be rejuvenated numerically through stock enhancement, a process in which large numbers of organisms are reared under artificial conditions and subsequently released into the natural environment (Leber 1995). Stock enhancement is receiving increasing attention as a management option, partly because its implementation appeals to our reluctance to impose harsher and less popular management or conservation methods (Travis et al. 1998). Additionally, stock enhancement approaches may create employment opportunities related to the construction and operation of hatchery facilities (Thacker 1994; Karney et al. 1997). In the United States, stock enhancement programs have been evaluated or are currently being evaluated in Alaska, California, Florida, Hawaii, Maine, Maryland, Massachusetts, Oregon, South Carolina, Texas, and Washington.

Perhaps due to growing popular interest, stock enhancement efforts often progress before questions inherent to this management approach are addressed (Munro and Bell 1997). From a feasibility perspective, two critical questions should be answered before stock enhancement is considered as a management option for a given species. First, is it possible to produce hatchery-reared (HR) organisms that are capable

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of surviving in the wild? Second, if so, can enhancement goals be met in a manner that is economically feasible? If these questions can be answered in the affirmative, then it is reasonable to further consider stock enhancement as a management option. However, responsible managers must also address ecological concerns prior to implementation of enhancement programs. Increasing evidence suggests that the mass release of HR organisms into the wild may have negative ecological ramifications, such as alteration of the genetic structure of natural populations (Busack and Currens 1995), displacement of natural populations with more aggressive HR conspecifics (e.g., Neilsen 1994), and impacts on the abundance and diversity of wild predators, prey, and competitors (Leber 1995).

Nested within the management concerns of stock enhancement are questions regarding the likely behavioural deficits of HR fish and their ability to survive in the wild. For example, natural cues and selection pressures for survival skills (e.g., ability to avoid predation or forage efficiently) are often bypassed in psychosensory-deprived laboratory conditions in which HR fish are initially raised (Olla et al. 1994, 1998; Ellis et al. 1997). The lack of selection pressures in hatchery environments may result in HR organisms that exhibit anomalous behaviours compared with wild conspecifics. If these anomalous behaviours, or behavioural deficits, result in HR organisms that are not behaviourally equipped to survive in the wild environment, then enhancement efforts will be of minimum value at best and futile at worst. Therefore, it is critical to address both the behavioural quality of HR fish and the ability of HR fish to survive in the natural environment before stock enhancement efforts proceed.

If HR fish exhibit abnormal behavioural patterns that result in decreased survival, and the abnormal behavioural patterns are not genetically based, then it may be possible to modify behavioural patterns to increase survival of HR fish in the wild. This idea has most often been approached from a predator-avoidance perspective (but see Olla et al. 1994; Zaragoza et al. 1994) and has been explored in several species of HR fish (e.g., rainbow and steelhead trout (*Oncorhynchus mykiss*) (Berejikian 1995; Brown and Smith 1998), coho salmon (*Oncorhynchus kisutch*) (Olla et al. 1994), and Atlantic salmon (*Salmo salar*) (Jaervi and Uglem 1993)). Inherent in this "antipredator conditioning" approach is the idea that HR fish may not be behaviourally equipped to deal with predators in the wild because of the predator stimulus free environment in which they have been reared. If HR fish can somehow be "conditioned" to recognize predators prior to release, then postrelease survival may be significantly increased.

Although much effort has been put into addressing behaviour and survival patterns of HR fish, scant information exists on North American flatfish species. Intense commercial and recreational exploitation, coupled with dwindling nursery habitat quality and area, makes summer flounder (*Paralichthys dentatus*) an excellent candidate for stock enhancement oriented studies (Waters 1996). The goals of this study were to assess the substrate-specific behavioural and survival capabilities of HR versus wild summer flounder and the feasibility of predator conditioning as a method to decrease susceptibility of HR individuals to predation.

Materials and methods

This study was conducted from February to July 1998 at the National Marine Fisheries Service laboratory in Beaufort, N.C., U.S.A. Newly hatched HR summer flounder yolk sac larvae were obtained from Great Bay Aquafarms, Inc. (New Hampshire) and raised to early juvenile stage (about 30–90 mm total length (TL)) in the laboratory. Rearing conditions were as documented by Burke et al. (1999), with the exception of water temperature, which ranged from 18 to 23°C for the fish used in this study. Wild young-of-the-year juveniles (about 30–90 mm TL) were collected from nearby shallow-water tidal flats in Beaufort, N.C., with a 2-m beam trawl (0.32-cm cod end mesh size). Wild fish were maintained in laboratory tanks for a minimum of 2 weeks prior to use in experimental trials to allow for dissipation of tidal rhythms (Morgan and Cordiner 1994) and for adjustment to general laboratory conditions. Because juvenile summer flounder utilize a spectrum of habitats as nursery areas, ranging from relatively low-energy mud substrate marsh habitats to relatively high-energy sand substrate beach habitats (Burke et al. 1991; T. Kellison et al., unpublished data), all experimental trials in this study were replicated on sand and mud substrates.

Behaviour of HR versus wild fish

Paired behavioural observations were made to compare substrate-specific behaviour of HR (28–63 mm TL) versus wild (34–69 mm TL) fish. In each behavioural trial, a single fish was introduced into one of three observation aquaria (22 cm length × 15 cm width × 15 cm depth) containing sand or mud substrate and screened from outside movement by black plastic. Following introduction, fish were left undisturbed for 24 h to acclimate to aquarium conditions. Each replicate fish was observed for 1 h through small openings in the plastic (two aquaria) or with a low-light sensitive video camera – monitor system (one aquarium). Light levels for daytime observations (about 1300–1400 lx) were based on field measurements. Nighttime behavioural observations were made with the video camera – monitor system using low-intensity red light (about 4–5 lx). To achieve paired (by time of day) observations, HR and wild fish were observed in adjoining 1-h time periods (i.e., back to back) (aquaria viewed through plastic screens) or with a 24-h interval between observations (aquarium viewed with the video camera). Paired behavioural observations began at a randomly chosen hour when using the video camera system, whereas paired observations made through the plastic screens by an observer were restricted to randomly chosen daylight hours. Statistical analyses indicated no effect of observation method (visual versus video camera system) on time spent buried, stationary on the benthos, or swimming in the water column (analysis of variance (ANOVA), $P > 0.24$ in all cases). Thus, data from the two observation methods were pooled for analyses. Because our focus was to identify possible behavioural differences between HR and wild fish, we did not address possible differences between diurnal and nocturnal behavioural patterns (although time-lapse video observations provide no evidence of differential circadian behavioural patterns between HR and wild fish).

To quantify substrate-specific behaviour of HR versus wild fish, fish activity was divided into four mutually exclusive categories: (1) buried, (2) stationary (not buried) on the benthos, (3) moving on the benthos, and (4) swimming in the water column. Behaviour was quantified by recording the amount of time during the 1-h observation period that each fish spent performing each of the four behaviours. Following each 1-h observation period, time spent performing each behaviour was summed within behavioural categories to calculate a total time spent performing each behaviour. Response variables were then calculated by subtracting the totals of HR fish from those of wild fish, by pair, within each behavioural category. Time spent moving on the benthos was excluded from

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statistical analyses for two reasons: (*i*) including all four behavioural categories would have nullified the statistical independence of the first three analyses (the four behaviours were mutually exclusive; the sum of the percent time spent performing the four behaviours for any observation was necessarily equal to 100%) and (*ii*) moving on the bottom was generally the most rarely exhibited behaviour, and therefore may be the least important in terms of vulnerability to predation. A total of 36 paired behavioural observations were made, split equally among substrate type (sand (n = 18) and mud (n = 18)). Behavioural data were analyzed using nonparametric statistics due to nonnormality of the response variables. A two-tailed Wilcoxon two-sample test (also referred to as a Mann–Whitney test) was first employed to test for an effect of substrate on time spent performing each behaviour. If there was no significant effect ($P > 0.05$) of substrate on the behavioural response of interest, we pooled data across substrates and used a Wilcoxon signed-rank test to determine whether the difference in time spent performing each behaviour between the HR and wild fish was significantly different from zero. One-tailed tests were employed for analyses of time spent buried and swimming in the water column, with the direction of difference dependent on the behaviour being analyzed. For example, we hypothesized that HR summer flounder would spend more time swimming in the water column than wild conspecifics based on behavioural observations of HR and wild Japanese flounder (*Paralichthys olivaceus*) (Furuta 1996, 1998). Similarly, we hypothesized that HR summer flounder would spend less time buried than wild conspecifics based on behavioural observations of HR and wild sole (*Solea solea*) (Howell and Baynes 1993; Ellis et al. 1997).

Cryptic adaptation to substrate

Experiments were performed to assess the ability of HR (34–77 mm TL) versus wild (35–79 mm TL) fish to become cryptic on a newly encountered substrate. Twelve 1.2-L containers (14 cm height, 12 cm diameter) were filled with seawater and equipped with 2 cm of natural substrate (sand (n = 6) or mud (n = 6)). Containers were placed in a flow-through water bath to keep temperatures similar to natural water temperatures (19–22°C). A single fish was then placed into each container in random order within a given substrate so that treatment combinations were orthogonal (HR–sand = 3, HR–mud = 3, wild–sand = 3, wild–mud = 3). Fish were observed immediately after introduction to the containers and subsequently observed at 5-min intervals for a 60-min period, whereupon each fish was assigned a value in minutes (0–60) corresponding to the elapsed time between introduction to the container and successful cryptic response. The "cryptic response" of each fish was quantified by recording the amount of time between introduction into the container and the successful completion of one of two behaviours: burial or pigmentation change to match the substrate. Successful burial was determined to occur when greater than 90% of a fish's body was covered in sediment. Successful pigmentation change was determined to have occurred when, at first glance following each 5-min interval, a fish was difficult to discern against the substrate background. The pigmentation and burial responses were therefore somewhat subjective but were designed as a "blind" test (the status (HR versus wild) of each fish was unknown to the observer) to ensure that potential observer bias did not affect results. Individuals that did not exhibit cryptic behaviour by trial termination were assigned a value of 61 min under the conservative assumption that a cryptic response would have occurred in the minute following trial termination. A total of 168 1-h trials were conducted, split equally among the four possible treatment combinations (HR–sand (n = 42), HR–mud (n = 42), wild–sand (n = 42), and wild–mud (n = 42)). Results were analyzed to determine the effect of fish status (HR versus wild) and substrate (sand versus mud) on both the time to cryptic response and the tendency to bury. After confirming homogeneity of variances, the time to cryptic response was analyzed using a two-way ANOVA with fish status (HR versus wild) and substrate (sand versus mud) as treatments. Although the data were not normally distributed, ANOVA is robust to deviations from normality, particularly as sample size increases (n = 42 in this case) (Zar 1984). Additionally, a two-way ANOVA on rank-transformed data (Akritas 1990) yielded P values nearly identical to those of the analysis of untransformed data, suggesting that the results of the parametric ANOVA were not compromised by the deviation from normality. A log-linear G test was used to determine whether fish status (HR versus wild) and substrate (sand versus mud) significantly affected the tendency of fish to bury. Comparisons between treatment combinations were determined with protected, lower-level chi square contrasts within the G test.

Antipredator conditioning and predation trials

The natural predator guilds of early juvenile (about 30–100 mm TL) summer flounder are relatively undocumented. Blue crabs (*Callinectes sapidus*) were chosen as the predator in this study because they are abundant in juvenile summer flounder nursery habitats (T. Kellison et al., unpublished data), they are documented omnivores, with piscivory often compiling a significant proportion of their diet (Stoner and Buchanan 1990) and they readily consumed summer flounder juveniles in the laboratory environment. Additionally, crabs are documented predators of several flatfish species (Ansell and Gibson 1993; Furuta 1996).

To test whether experience with predator cues could increase survival rates of HR fish, 53 of the 106 HR fish (46–72 mm TL) used in predation trials (see below) were exposed to a single caged blue crab (>100 mm carapace width) for 24 h prior to predation trials. The caged crab was placed in the center of a tank containing HR fish and was fed juvenile spot (*Leiostomus xanthurus*) once during the 24-h antipredator conditioning period. Fish that received the antipredator conditioning treatment are subsequently referred to as antipredator-conditioned HR (APCHR) fish as opposed to HR fish.

Predation trials were conducted to test whether fish status (HR versus APCHR versus wild) and substrate (sand versus mud) had a significant effect on summer flounder mortality due to predation. Predation trials were conducted in 12 individually aerated aquaria (46 cm length × 23 cm width × 25 cm depth) with flow-through seawater and either sand (n = 6) or mud (n = 6) substrate. Each aquarium was screened from outside movement with black plastic. Fish were randomly assigned to a tank (within substrate) and allowed to acclimate to tank conditions for 24 h. At the end of the 24-h acclimation period, a blue crab (80–110 mm carapace length) was placed in the tank. Tanks were then left undisturbed for a 24-h period, after which crabs were removed from each tank and fish from each trial classified as "eaten" or "not eaten." Predation trails were replicated within fish status treatment levels (HR versus APCHR versus wild) on both sand (n = 33 per treatment) and mud (n = 20 per treatment) for a total of 159 trails. Data were analyzed using a log-linear G test with fish status (HR versus APCHR versus wild) and substrate (sand versus mud) as categories.

Results

Behaviour

Paired behavioural trials

Behavioural patterns of HR fish generally differed from those of wild conspecifics (Fig. 1). HR fish spent significantly more time swimming in the water column than wild conspecifics (one-tailed Wilcoxon signed rank test; data pooled over substrate (see below): $T_{35} = -78$, $P = 0.011$)

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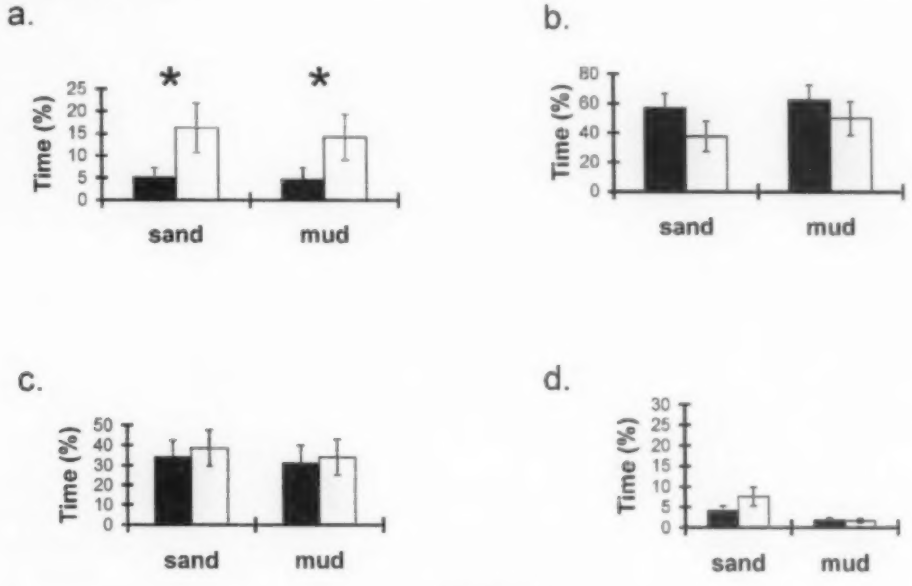
Fig. 1. Mean percent time (±SE) spent (*a*) swimming in the water column, (*b*) buried, (*c*) stationary (not buried) on the benthos, and (*d*) moving on the benthos by wild (solid bars) and HR (open bars) juvenile summer flounder. Statistical analyses were based on paired observations (Wilcoxon signed-rank test: $\alpha = 0.05$). Asterisks denote significant differences between the HR and wild responses, pooled over substrates; $n = 18$ paired observations for each behaviour divided equally between substrates (sand ($n = 9$) and mud ($n = 9$)). See text for significance levels.



(Fig. 1*a*). HR fish spent less time buried than wild fish (Fig. 1*b*), although the mean difference in time spent buried between HR and wild fish was not statistically significant (one-tailed Wilcoxon signed rank test; data pooled over substrate: $T_{35} = 46$, $P = 0.067$). There was no significant difference in the amount of time spent stationary (not buried) on the benthos between HR and wild fish (one-tailed Wilcoxon signed rank test; data pooled over substrate: $T_{35} = 1$, $P = 0.491$) (Fig. 1*c*). Substrate (sand versus mud) had no significant effect on time spent by HR and wild fish swimming in the water column (Wilcoxon two-sample test: $Z = 0.322$, $P = 0.747$) (Fig. 1*a*), buried (Wilcoxon two-sample test: $Z = 0.147$, $P = 0.883$) (Fig. 1*b*), or stationary (not buried) (Wilcoxon two-sample test: $Z = 0.095$, $P = 0.924$) (Fig. 1*c*).

Cryptic adaptation to substrate

HR fish suffered an impaired ability to exhibit cryptic behaviours on a newly encountered substrate compared with wild conspecifics. HR fish took significantly longer than wild fish to become cryptic (burial or pigmentation response) on the benthos, regardless of substrate (two-factor ANOVA with interaction term; treatments = fish status (HR versus wild) and substrate (sand versus mud): $F_{1,164} = 42.91$, $P < 0.0001$) (Fig. 2*a*). Time to cryptic response was significantly longer on mud than on sand substrate, regardless of fish status (two-factor ANOVA with interaction term; treatments = fish status and substrate: $F_{1,164} = 3.93$, $P = 0.049$) (Fig. 2*a*). The fish status × substrate interaction was not significant (two-factor ANOVA with interaction term: $F_{1,164} = 0.43$, $P = 0.514$). HR fish buried significantly less often than wild fish, regardless of substrate (log-linear G test with interaction term; treatments = fish status and substrate: $\chi^2 = 8.9980$, $P = 0.003$) (Fig. 2*b*). Additionally, substrate had a significant effect on the tendency of fish to bury. Both HR and wild fish buried significantly more often on sand than on mud (log-linear G test with interaction term; treatments = fish status and substrate: $\chi^2 = 23.8381$, $P < 0.0001$) (Fig. 2*b*). The fish status × substrate interaction was not significant (log-linear G test with interaction term: $\chi^2 = 0.22$, $P = 0.636$).

Predation and antipredator conditioning

HR fish exhibited greater susceptibility to predation than wild conspecifics. Increased vulnerability of HR fish to predation was partially mitigated by predator conditioning. Fish survival varied significantly according to fish status (HR versus APCHR versus wild) (log-linear G test with interaction term; treatments = fish status and substrate: $\chi^2 = 22.51$, $P < 0.0001$). HR fish suffered significantly greater predation rates than wild fish (protected contrast within log-linear G test: $\chi^2 = 22.46$, $P < 0.0001$) (Fig. 3). APCHR fish experienced significantly lower predation rates than HR fish (protected contrast within log-linear G test: $\chi^2 = 4.33$, $P = 0.038$) but experienced significantly greater predation rates than wild fish (protected contrast within log-linear G test: $\chi^2 = 7.67$, $P = 0.006$) (Fig. 3). Substrate had no significant effect on summer flounder mortality due to predation, regardless of fish status (log-linear G test with interaction term; treatments = fish status and substrate: $\chi^2 = 0.20$, $P = 0.658$) (Fig. 3).

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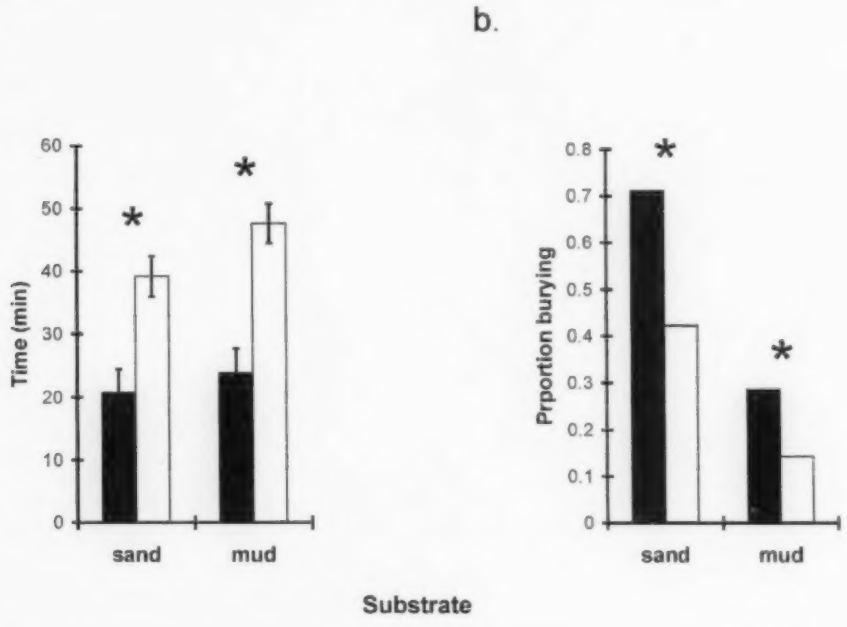
Fig. 2. (*a*) Mean time (±SE) required to become cryptic (burial or cryptic pigmentation) on the benthos by wild (solid bars) and HR (open bars) summer flounder. Asterisks denote significant differences between the HR and wild responses, regardless of substrate (two-factor ANOVA with interaction term; treatments = fish status and substrate: $\alpha = 0.05$, $n = 180$). (*b*) Proportion of wild (solid bars) and HR (open bars) fish that exhibited burying behaviour during the cryptic adaptation trials. Asterisks denote significant differences between the HR and wild responses, regardless of substrate (log-linear G test with interaction term; treatments = fish status and substrate: $\alpha = 0.05$, $n = 180$).



There was no significant fish status × substrate interaction (log-linear G test with interaction term: $\chi^2 = 4.26$, $P = 0.119$).

Discussion

Behaviour and predation

The behavioural data in this study suggest that (*i*) HR summer flounder exhibit modified behavioural patterns relative to wild conspecifics, (*ii*) these modified behavioural patterns reduce survival in laboratory aquaria, and (*iii*) decreased survival of HR summer flounder can be partially mitigated by exposing HR fish to a predator stimulus prior to their exposure to a predator. Flatfish are evolutionarily specialized for a benthic existence. Their body form allows them to lie flat on the benthos, and natural behaviours such as burial and mimicry of the benthos are important to survival, increasing the ability to successfully ambush prey and elude the detection of potential predators (Ansell and Gibson 1993; Howell and Baynes 1993). HR fish in this study spent less time buried, were significantly less likely to bury, and took significantly longer amounts of time to become cryptic on the benthos than wild fish.

While the ability to bury and mimic benthic coloration and pattern are innate in HR summer flounder, such behaviours were poorly utilized compared with wild conspecifics and thus constitute "behavioural deficits" (Olla et al. 1994). Lack of experience on natural substrates may be a key factor causing behavioural deficits related to becoming cryptic on the benthos in this study. Previous research with flatfish has suggested that behavioural deficits may diminish with exposure to natural conditions or stimuli. For example, both burying efficiency (the proportion of the ocular side covered by sand after a single burial attempt) and the ability to change pigmentation improved significantly with time spent on natural substrate in HR sole (Ellis et al. 1997). Other possibilities explaining the lack of ability to become cryptic on natural substrate by HR fish include hatchery diet deficiencies, which may preclude the development of normal pigmentation abilities (e.g., Seikai 1985), and stress, which may be a combination of many factors present in the hatchery environment, including diet deficiencies. If lack of experience on natural substrates is the main causative agent of the lack of ability of HR fish to become cryptic on natural substrates, then the use of rearing facilities that utilize natural substrate may be a critical step towards rearing hatchery fish that are behaviourally equipped to survive in the wild. Unfortunately, high-density culture techniques, such as those used in the rearing of summer flounder, necessitate high feeding rates and frequent cleaning of holding tanks, making use of natural substrates difficult and problematic. If additional factors, such as diet deficiencies, general stress, or selection for fish that are adapted to hatchery conditions, are the causes of the disability of HR fish to become cryptic on natural substrates, then alternative rearing strategies (e.g., improved diets or reduced-stress environments) must be employed or developed if HR fishes are to be equipped behaviourally to survive in the wild.

While cryptic behaviours such as burial and benthic mim-

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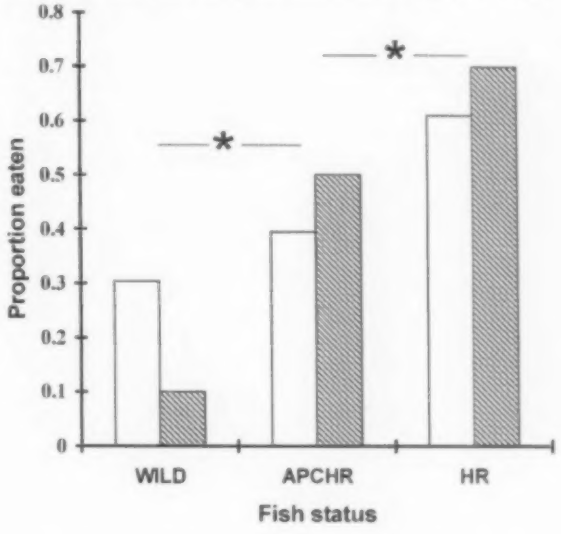
icry are intuitively adaptive for flatfish, behaviours that make summer flounder more visible to predators, such as swimming in the water column, are intuitively maladaptive. The HR fish in this study spent significantly greater amounts of time swimming in the water column than wild conspecifics. This behaviour may be a result of hatchery feeding techniques, in which food pellets are dropped into rearing tanks. Fish that learn to swim towards the surface are rewarded with abundant food under such a feeding regime. Thus, swimming in the water column is rewarded (and therefore reinforced) and possibly selected for in the hatchery environment. Upon release, however, this behaviour would quickly become detrimental to survival. For example, laboratory experiments and field observations of Japanese flounder indicated that HR juveniles suffered high rates of predation-induced postrelease mortality due to increased amounts of time spent swimming in the water column as compared with wild fish (Furuta 1996). It is likely that increased activity, especially with regard to swimming in the water column, was a significant factor affecting the increased susceptibility of HR summer flounder to predation in this study (see below). If common hatchery feeding techniques (such as those employed in the rearing of the experimental HR fish used in this study) result in HR flatfish conditioned to swimming in the water column, then such aberrant behaviour may be altered by developing and utilizing alternative feeding techniques in which food is introduced on the bottom of rearing tanks (e.g., Berejikian et al. 1999).

HR fish in this study suffered significantly higher predation rates than wild conspecifics, regardless of substrate. It is probable that HR fish were more susceptible to predation due to a combination of modified behavioural patterns (increased time spent swimming in the water column, decreased time spent buried or stationary on the benthos, and decreased ability to exhibit cryptic behaviour on the benthos compared with wild conspecifics). Although predation by blue crabs is often regarded as dependent on tactile and chemical cues (Eggleston 1990; Weissburg and Zimmer-Faust 1994), predator–prey interactions observed in preliminary predation trials appeared to have been visually mediated (when a fish displayed movement, e.g., swimming into the water column, crabs immediately directed their attention toward the fish (chelipeds spread with chelae open) and subsequently pursued the fish). If visual cues are a significant contributor to the initiation of crab – summer flounder predator–prey interactions, then the behavioural deficits exhibited by HR fish in this study (more time spent swimming in the water column and less time cryptic on the benthos) are a plausible mechanism underlying the increased predation rates on HR summer flounder. If HR summer flounder are significantly more susceptible to blue crab predators than wild conspecifics, then it is likely that HR summer flounder would also suffer increased susceptibility to predation from other visual predators, such as piscivorous fish. Therefore, the aberrant behaviours exhibited by naïve HR summer flounder are likely to result in abnormally high rates of predation-induced mortality in the natural environment.

The APCHR fish experienced significantly lower predation rates than naïve HR fish but suffered significantly higher predation rates than wild fish in the predation trials. These results add to the growing evidence that deficiencies

Fig. 3. Proportion of summer flounder (wild, APCHR, and naïve HR) eaten by blue crabs in laboratory predation trials on sand (open bars) and mud (hatched bars) substrates. The asterisks bordered by lines denote significant differences between the wild, APCHR, and HR responses (log-linear G test with interaction term; treatments = fish status and substrate: $\alpha = 0.05$, $n = 159$).



of HR fish, perhaps manifest in abnormal behavioural patterns, can be partially mitigated by exposing HR fish to natural predator cues prior to their release (antipredator conditioning) (Olla et al. 1994; Brown and Smith 1998). The mechanisms underlying the decrease in predation rates on APCHR fish compared with naïve HR fish are unknown. If the APCHR fish learned to recognize the blue crab to which they were being conditioned as a predator, they may have altered their behaviour by decreasing motion in the presence of a predator (i.e., during predation trials). Further research is required to determine: what combination of cues results in predator recognition and possibly antipredator behaviour and how antipredatory behaviour is manifest in summer flounder. Such research will aid in determining the best methods by which to condition HR summer flounder for release into natural environments.

Mechanisms underlying modified behavioural patterns

HR fish may exhibit modified behavioural patterns due to three nonmutually exclusive mechanisms: (*i*) genetic differences from wild populations (Reisenbichler and Brown 1995; Petersson et al. 1996), (*ii*) a lack of appropriate natural behavioural cues (Olla et al. 1994, 1998; Berejikian 1995), or (*iii*) a lack of natural selection pressures in the psychosensory-deprived hatchery environment (Berejikian 1995; Olla et al. 1998). The behavioural deficits documented in this study are most likely not genetically based, as the multiple broodstock were captured from the wild as adults. It is likely that a lack of appropriate natural behavioural cues contributed to the modified behavioural patterns exhibited by HR summer flounder in this study, as suggested by the fact that naïve HR fish were significantly more susceptible to predation than antipredator-conditioned fish. Additionally, a

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lack of natural selective pressures in the hatchery environment may have been an important factor affecting the increased susceptibility of HR fish to predation in this study. The wild fish with which the HR fish were compared in this study are assumed to have passed the "tests" of natural selection prior to collection. In such tests, wild fish that exhibit detrimental behavioural patterns (i.e., lack of ability to become cryptic on the benthos and a high proportion of time spent swimming in the water column) have presumably perished due to predation. Thus, the wild fish used in this study were "winners" in the game of natural selection thus far, while the HR fish, which have experienced no selective predation, remain a mixture of would-be winners and losers. From this viewpoint, the high postrelease mortality rates often suffered by HR fish are simply ridding the HR population of behaviourally deficient "losers." Such observations have prompted suggestions of including natural predators in hatchery-rearing programs (Independent Scientific Group 1999). Such novel approaches may be necessary to mitigate the effects of behavioural deficits, which may be responsible for the elevated levels of postrelease predation-induced mortality common to HR fishes (e.g., see Furuta 1996). Nevertheless, approaches designed to mitigate the effects of behavioural deficits on postrelease survival may add significant cost and effort to the rearing of HR organisms and should therefore be thoroughly examined in terms of economic and logistical feasibility prior to implementation.

Substrate

Previous research with estuarine-dependent fishes indicates that habitat is extremely important in determining growth and survival (Hoss and Thayer 1993). Thus, it may be critical to assess habitat-specific behaviour and survival of stock enhancement candidates to make informed decisions about optimal habitats for release. Substrate, representative of beach and marsh habitat in this study, was an important factor affecting the likelihood of fish to bury. Previous work with the closely related Japanese flounder suggests that flatfish exhibit increased burying behaviour on preferred substrate (Tanda 1990), which has been identified as sand for juvenile summer flounder (Keefe and Able 1994). In support of Tanda's (1990) suggestion of increased burial on preferred substrate, both HR and wild fish in this study took significantly less time to exhibit cryptic behaviour (pigmentation response or burial) and were significantly more likely to bury on sand than on mud substrate. These results also support the conclusions of Keefe and Able (1994), who reported that metamorphic (about 15 mm standard length) summer flounder buried significantly more often in sand than in mud. If HR fish exhibit increased burying activity in sand substrate habitats in the wild, and if burial enhances survival, then these results suggest that sand substrate habitats may support greater survival of juvenile summer flounder than mud substrate nursery habitats. This information has important implications for release of HR summer flounder in the wild (i.e., optimal release habitat) and should be tested in the field.

In all other cases, substrate had no significant effect on behaviour or susceptibility to predation. These results suggest that if differences exist in habitat-specific behaviour and survival, then, aside from cryptic abilities (pigmentation response or burial), these differences are not a result of substrate but of other habitat-specific factors. Such factors may include both biotic (prey availability and predator guilds) and abiotic (differences in temperature, salinity, dissolved oxygen, etc.) aspects of particular habitats. Habitat-specific biotic and abiotic factors likely interact at many levels to affect behaviour, growth, and survival of flatfish and other estuarine-dependent fishes (Keefe and Able 1994; Burke 1995).

In summary, the results of this study suggest that HR summer flounder, reared in the psychosensory-deprived hatchery environment, are not well equipped to survive in natural environments compared with wild conspecifics. The modified behavioural patterns (behavioural deficits) exhibited by HR fish in this study may be a mechanism underlying the reduced ability of HR fish to withstand predation in laboratory trials. Importantly, HR fish exposed to natural predator stimuli exhibited increased survival rates in this study, suggesting that behavioural deficits and, more importantly, increased susceptibility to predation can be mitigated through predator conditioning. The empirical research described herein may serve as a framework for evaluating the behavioural and survival capabilities and the ability to mitigate behavioural and survival deficits for other stock enhancement candidate species. The type of experimental approach used in this study, which specifically addresses stock enhancement feasibility, is an essential component of a responsible and well-rounded approach to stock enhancement (Leber 1995).

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A fuzzy logic model with genetic algorithm for analyzing fish stock–recruitment relationships

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Abstract: A new fuzzy logic model with a genetic algorithm is developed that overcomes some of the inherent uncertainties in the fish stock–recruitment process. This model is applied to stock–recruitment relationships for the Southeast Alaska pink salmon (*Oncorhynchus gorbuscha*) and the West Coast Vancouver Island Pacific herring (*Clupea pallasi*) stocks. In both examples, the annual mean sea surface temperature is used as an environmental intervention in the model. The fuzzy logic model provides the functional relationship between the number of fish spawners and the sea surface temperature that is used to reconstruct the historical fish recruitment time series and also to predict the number of fish that will recruit in the future. Globally optimized genetic learning algorithms are used to find the optimal values of the parameters for the fuzzy logic model. The results from this fuzzy logic model are compared with results from both a traditional Ricker stock–recruitment model and a recent artificial neural network model. These comparisons demonstrate the superior capability of the fuzzy logic model for addressing problems of uncertainty and vagueness in both the data and the stock–recruitment relationship. The fuzzy logic model approach is recommended as a useful addition to the analytical tools currently available for fish stock assessment and management.

Résumé : Nous avons élaboré un nouveau modèle à logique floue avec algorithme génétique qui contourne certaines des incertitudes inhérentes au processus de recrutement des stocks de poissons. Nous appliquons ce modèle aux relations stock–recrutement pour les stocks de saumon rose du sud-est de l'Alaska et de hareng de la côte ouest de l'île de Vancouver. Dans les deux exemples, la moyenne annuelle de la température de la surface de la mer représente l'intervention de l'environnement dans le modèle. Le modèle à logique floue fournit la relation fonctionnelle entre le nombre de géniteurs et la température de la surface de la mer qui sert à reconstituer la série chronologique historique du recrutement des poissons, et aussi à prédire le nombre de poissons qui vont recruter dans l'avenir. Nous avons recours à des démarches d'apprentissage génétique globalement optimisées pour trouver les valeurs optimales des paramètres pour le modèle à logique floue. Les résultats obtenus par ce modèle sont comparés à ceux d'un modèle traditionnel stock–recrutement de Ricker et à ceux d'un modèle récent de réseau neuronal artificiel. Ces comparaisons font ressortir la capacité supérieure du modèle à logique floue pour régler les problèmes d'incertitude et d'imprécision tant dans les données que dans la relation stock–recrutement. L'approche du modèle à logique floue nous semble un ajout utile à la panoplie des outils d'analyse qui existent actuellement pour l'évaluation et la gestion des stocks.

[Traduit par la Rédaction]

Introduction

The analysis of stock–recruitment relationships is frequently an important step in developing or evaluating fishery policies, such as establishing optimal escapement goals for salmon or optimal size of spawning stocks for fish species that reproduce in the ocean. Traditional stock–recruitment analyses are based on the assumption that a functional relationship exists between the size of the spawning stock and the number of fish that recruit in the future. If they are included at all, the effects of changing environmental conditions and human interventions (e.g., harvest by fisheries) on the stock–recruitment parameters are typically estimated using least-squares or maximum likelihood methods (Ricker 1975; Hilborn and Walters 1992; Quinn and Deriso 1999).

Three major sources of error are embedded in these traditional stock–recruitment analyses. The first source is measurement error that arises from the collection of the data in the field or from experiments. This type of error is commonly referred to as the "errors-in-variables" and has been closely examined (Ludwig and Walters 1981; Walters and Ludwig 1981; Hilborn and Walters 1992).

The second source of error arises from the assumptions made about processes in the analytical model. The Ricker (1975) model has traditionally been widely used to characterize the stock–recruitment relationship for fish in both marine and freshwater populations. Alternative stock–recruitment models have been described by Beverton and Holt (1957), Schnute and Kronlund (1996), and others. Application of these various models to the same stock–recruitment data frequently yields substantially different results.

The third source of error results from the "fit" of the analytical model to the data. Even if the correct model is identified and the data are collected without error, the methods

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that the model uses to fit the data may produce errors in the parameter estimates. This situation is most severe when there are many local minima or maxima in the stock–recruitment parameter space.

The numerous analytical approaches developed to attempt to resolve these difficulties (e.g., nonlinear regression, likelihood method, Bayesian analysis, bootstrapping, and nonparametric methods) have been reviewed by Hilborn and Walters (1992) and Quinn and Deriso (1999).

Recently, there has been increasing interest in applying machine learning algorithms such as artificial intelligence (AI) neural network models to stock–recruitment analyses (e.g., Lek et al. 1995; Aoki and Komatsu 1997; Guegan et al. 1998). Saila (1996) provided a comprehensive guide for the application of AI models, including neural network and fuzzy logic, to research and management of fisheries and oceans. This approach appears to offer substantial advantages and improvements to the more traditional methods used to analyze stock–recruitment relationships. A neural network model frequently provides a better fit to the data and improved recruitment forecasts because there are fewer constraints on the assumed form of the functional relationships between recruitment, spawning stock, and other environmental or human intervention variables (e.g., Jarre-Teichmann et al. 1995; Chen and Ware 1999; Mackinson et al. 1999). Two of the most attractive features of the genetic algorithm approach are that it appears to be widely applicable and the search procedure for the model parameters is very efficient.

Similar to the AI neural network, fuzzy logic models are designed to mimic how the human brain tends to classify some information or data imprecisely, such as concluding that water temperatures are "cool," "warm," or "hot." In fuzzy logic models, information is processed in terms of fuzzy sets. The fuzzy set is made precise through the definition of an associated membership function. The specific inference is then processed by the fuzzy set combined with some fuzzy rules (e.g., "if the number of fish spawners is high, and the sea surface temperature is cold, then the fish recruitment is high"). The fuzzy logic model combines one or more input signals, which are defined by the fuzzy sets, with a collection of fuzzy rules to produce an output that can be compared with the actual values observed in the real world.

Similar to the AI neural networks, fuzzy logic systems can be used to model nonlinear relationships. Unlike neural networks, however, fuzzy logic systems can provide insight into their own operation because the fuzzy rules provide an easily understood and common sense description of the action of the fuzzy logic system. In this sense, the fuzzy logic system complements the neural network approach for dealing with complex nonlinear problems. A fuzzy logic system is a well-defined function that maps real-value inputs to real-value outputs. Recent results have also shown that fuzzy logic systems are universal approximators (Kosko 1993) for general nonlinear functional relationships, to any desired degree of accuracy. This makes fuzzy logic modelling a powerful tool for exploring complex, nonlinear biological problems like stock–recruitment analyses and forecasting.

In this paper, a fuzzy logic model is developed to analyze the stock–recruitment relationship, including the effects of an environmental variable. The parameters in this model are estimated using a genetic algorithm that minimizes the root mean square error (RMSE) between the observed and modelled recruitment. This new fuzzy logic model with genetic algorithm (hereafter called the Fuzzy–GA model) (*i*) characterizes the uncertainties in the data (the first source of error) by fuzzification of each type of stock–recruitment data into a fuzzy set with associated degrees of membership data (the second source of error), (*ii*) provides a functional or data-driven approximation to the underlying theoretical model, (*iii*) provides estimates of the model parameters that are guarantee to be optimized (the third source of error), and (*iv*) provides a functional relationship that is fitted from the historical stock–recruitment data and can be used to forecast future recruits even if the input data are uncertain or vague (e.g., the size of the spawning stock can be characterized as either "small" or "large" but cannot be quantified more precisely). The performance of the Fuzzy–GA model is compared with the results from a traditional Ricker stock–recruitment model and also with the neural network model proposed by Chen and Ware (1999). Two real data sets, one for pink salmon (*Oncorhynchus gorbuscha*) from Southeast Alaska (SEAK), U.S.A., and the second for Pacific herring (*Clupea pallasi*) from West Coast Vancouver Island (WCVI), British Columbia, Canada, are evaluated.

Methods

Fuzzy logic model

A fuzzy logic model is also known as a fuzzy inference system or a fuzzy rule based system. Basically, any fuzzy logical model constitutes three parts: the fuzzy membership function, fuzzy decision rules, and fuzzy reasoning. In this paper, a fuzzy logic model similar to that described by Takagi and Sugeno (1983) and Liu and Kojima (1993) was used to develop a fuzzy logic model of fish stock–recruitment. There are two input variables in this fuzzy logic model: x_1 is the biomass of spawners (i.e., parents) in the brood year in which the recruits were born and x_2 is the annual mean sea surface temperature (SST) in the same brood year. The output of fuzzy logic model is y, which is the biomass of fish recruits that results from the corresponding spawning in brood year t.

Membership function

The membership function for the input variables x_1 (spawner biomass) and x_2 (SST) is shown in Fig. 1. The values of k in the figure for both input variables (k_1 and k_2) are equal to the mean values of each time series. Using these mean (k) values, the data for the two input variables were classified into three linguistic categories: "small," "middle," or "large" for spawner biomass and "low," "middle," or "high" for SST. The real data for both the SST and spawner biomass are actually much more quantitative than is reflected by these three categories. However, the advantage of using these categories is that we think this more accurately reflects the actual level of uncertainty that is inherent in these data, especially for spawner biomass. For example, estimates of fish spawning biomass tend to be highly variable, and even knowledgeable experts might debate and fail to reach agreement about the "true" value of the spawning biomass for any given year. However, they would likely easily agree on whether the spawning biomass was small, middle (average), or large relative to the long-term average.

Fuzzy rules

There are nine fuzzy rules that are totally determined by the choice of the fuzzy membership function defined in Fig. 1. In general form, each fuzzy rule is written as

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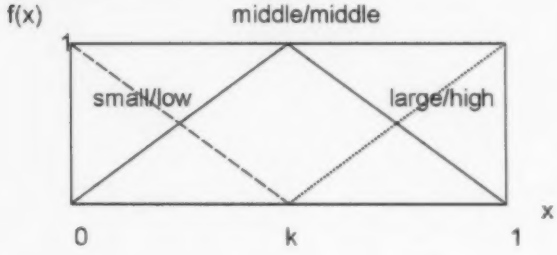
Fig. 1. Example of the membership function for the input variables (spawner biomass and SST). The parameter k in each case is chosen to be the mean value of the input variable to classify the input into three fuzzy sets ("small," "middle," and "large" for spawner input and "low," "middle," and "high" for SST input). In the figure, the dashed line is the membership function for the fuzzy "small/low" set, the dotted line on the right is the membership function for the fuzzy "large/high" set and the middle solid lines are the membership function for the fuzzy "middle/middle" set.



Rule: if x_1 is A_1 and x_2 is A_2,

$$\text{then } y = ax_1 + bx_2 + c$$

where A_1 and A_2 are the fuzzy sets that describe the nature of the inputs, such as "small," "medium," or "large," and a, b, and c are the parameters that are to be estimated using the genetic algorithms. Therefore, the "consequent" parts of the nine fuzzy rules are defined by the nonfuzzy equations of the two input variables (x_1 and x_2) as follows:

Rule 1: if x_1 is small and x_2 is low, then

$$y_1 = a_1x_1 + b_1x_2 + c_1$$

Rule 2: if x_1 is small and x_2 is middle, then

$$y_2 = a_2x_1 + b_2x_2 + c_2$$

Rule 3: if x_1 is small and x_2 is high, then

$$y_3 = a_3x_1 + b_3x_2 + c_3$$

Rule 4: if x_1 is middle and x_2 is low, then

$$y_4 = a_4x_1 + b_4x_2 + c_4$$

Rule 5: if x_1 is middle and x_2 is middle, then

$$y_5 = a_5x_1 + b_5x_2 + c_5$$

Rule 6: if x_1 is middle and x_2 is high, then

$$\text{then } y_6 = a_6x_1 + b_6x_2 + c_6$$

Rule 7: if x_1 is large and x_2 is low,

$$y_7 = a_7x_1 + b_7x_2 + c_7$$

Rule 8: if x_1 is large and x_2 is middle, then

$$y_8 = a_8x_1 + b_8x_2 + c_8$$

Rule 9: if x_1 is large and x_2 is high, then

$$y_9 = a_9x_1 + b_9x_2 + c_9$$

where a_i, b_i, and c_i (i = 1 to 9) are the fuzzy parameters to be estimated from the genetic algorithms.

Fuzzy reasoning

Suppose that we have the above "implications" Rule i (i = 1 to 9) and we are also given that ($x_1 = X_1$, $x_2 = X_2$), where X_1 and X_2 are the observed values of x_1 (the fish spawner biomass) and x_2 (the observed annual SST). The model value of y is then inferred from the following steps.

Step 1: For each implication Rule i, y_i is calculated by the function g_i in the consequence (Takagi and Sugeno 1983)

$$y_i = g_i(X_1, X_2) = a_iX_1 + b_iX_2 + c_i.$$

Step 2: The truth value of the "proposition" $y = y_i$ is calculated by the equation

$$|y = y_i| = |X_1 \text{ is } A_{i1}, X_2 \text{ is } A_{i2}|\text{^}|R_i| = (A_{i1}(X_1)\text{^}A_{i2}(X_2))\text{^}|R_i|$$

where $|*|$ means the truth value of proposition "*" and "^" stands for min operation and $|X \text{ is } A| = A(X)$, i.e., the grade of the membership of X. For simplicity, we assume that $|R_i| = 1$, so the truth value of the consequence obtained is

$$|y = y_i| = A_{i1}(X_1)\text{^}A_{i2}(X_2).$$

Step 3: The final output y that is inferred from the nine implications is given as the average of all y_i with the weights $|y = y_i|$:

$$y = \frac{\sum |y = y_i| \times y_i}{\sum |y = y_i|}$$

Table 1 shows the reasoning process for each implication when we are given $x_1 = X_1$ and $x_2 = X_2$. The column "Premise" in Table 1 shows the membership functions of the fuzzy sets "small," "middle," and "large" for the spawner biomass variable and "low," "middle," and "high" for the SST in the premises. The column "Consequence" shows the value of y_i calculated by the function g_i of each consequence for the inputs and corresponding parameters, and the column "Weight" is obtained from the fuzzy "and" operation for the memberships of the inputs. Finally, the fuzzy logic model output value inferred by the implications is obtained as follows:

$$(1) \qquad y = \frac{\sum_{i=1}^{9} y_i\alpha_i}{\sum_{i=1}^{9} \alpha_i}$$

A numerical example of the fuzzy reasoning process using data from the WCVI Pacific herring stock is given in the Appendix.

Genetic learning optimization algorithm

In the above section, we discussed how to construct a fuzzy logic model that can be used to estimate recruitment from various input data. The consequent-part parameters (a_i, b_i, c_i) in the fuzzy logic model depend on the input–output data and usually can be identified by any mathematical minimization procedure. However, it is difficult to find the optimal set of consequent-part parameters when there are a large number that have to be estimated (27 in this case). In such instances, conventional mathematical search algorithms are likely to converge on some local minimum instead of the global minimum. In this paper, we use genetic algorithms to find the optimal consequent-part parameters. Beasley et al. (1993) discussed the advantages of using genetic algorithms in multimodal function optimization. In his programming guide in C/C++, Welstead (1994) compared the genetic algorithm with the most commonly used optimization algorithms, such as the steepest descent and the conjugate gradient method, and concluded that the

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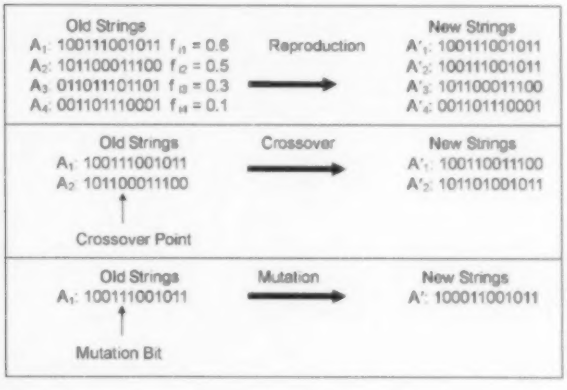
Table 1. Fuzzy reasoning process.

Implication Premise		Consequence	Weight α
R_1	small, low	$y_1 = a_1X_1 + b_1X_2 + c_1$	$\alpha_1 = 0.4 \wedge 0.5 = 0.4$
R_9	large, high	$y_9 = a_9X_1 + b_9X_2 + c_9$	$\alpha_9 = 0.6 \wedge 0.5 = 0.5$
	$x_1 = X_1$	$x_2 = X_2$	

Note: "Implication premise" describes the fuzzy membership function for the two inputs spawner abundance (or biomass) and SST under nine fuzzy rules, "Consequence" is the value calculated from each consequence for the inputs and corresponding parameters, and "Weight α" is calculated from the fuzzy "and" operation for the memberships from the two inputs.

Fig. 2. Example of three genetic algorithm operators: reproduction, crossover, and mutation. The string in this case represents one possible combination of all the model parameters.



genetic algorithm is a global optimization algorithm. Detailed discussions of genetic algorithms can be found in Karr (1991) and Liu and Kojima (1993).

Genetic algorithms

A genetic algorithm is a searching algorithm based on the mechanics of natural selection and genetics. A genetic algorithm combines the "survival of the fittest" concept among string structures with a structured yet randomized information exchange to form a search algorithm with some of the innovative flair of human search. In every generation, a new set of strings is created using bits and pieces of the "fittest" strings of the previous generations. While randomized, a genetic algorithm does not perform a random walk through the search space. Instead, it uses random choice efficiently in its exploitation of prior knowledge to locate optimal solutions rapidly. A genetic algorithm differs from more conventional search techniques because it considers many points in the search space simultaneously and therefore has a reduced chance of converging to some local optima.

A genetic algorithm utilizes three fundamental "genetic" operators: reproduction, crossover, and mutation (Fig. 2). Reproduction is a process where strings with large fitness value (i.e., good solutions to the problem at hand) receive correspondingly large numbers of copies in the new population. Crossover provides a mechanism for strings to mix and match their desirable qualities through a random process. The crossover process occurs in three steps. First, two newly reproduced strings are selected. Second, a position along the two strings is selected at random. The third step is to exchange all characters after the selected crossover position. Finally, mutation is the occasional alteration of a value at a particular string position. It is an insurance policy against the permanent loss of any simple bit and enhances the ability of the genetic algorithm to find the optimal solution. By analogy with a biological genetic system where mutations are rare, a higher probability (0.6) is given to the crossover process than to the mutation process (0.005).

Learning algorithm

A common "concatenated mapped, unsigned binary coding" is used to code the consequent parameters of fuzzy logic model. For example, a parameter p, which is the consequent parameter of the fuzzy logic model, is discretized by mapping from a minimum value p_{min} to a maximum value p_{max} using an m-bit, unsigned binary integer according to the equation

$$p = p_{min} + (p_{max} - p_{min}) \times \frac{b}{2^m - 1}$$

where b is the integer value represented by an m-bit string (for this application, $m = 8$). All the parameters are coded using the same method. Accordingly, all of the parameters needed to construct a fuzzy logic model can be represented by one long string with length 216 bits (total 27 parameters with $m = 8$ bits for each parameter).

A fitness function (a term used in the genetic algorithm, which acts like an objective function in any optimization problem) is required to apply the genetic algorithm. We use the RMSE between the observed recruit numbers (or biomass) and the output from the fuzzy logic model as the fitness function. Figure 3 illustrates the steps and data flow in the genetic learning algorithm. As an example, Fig. 4 shows the change in the fitness function (RMSE) in sub-

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Fig. 3. Genetic learning algorithm program and data flow.

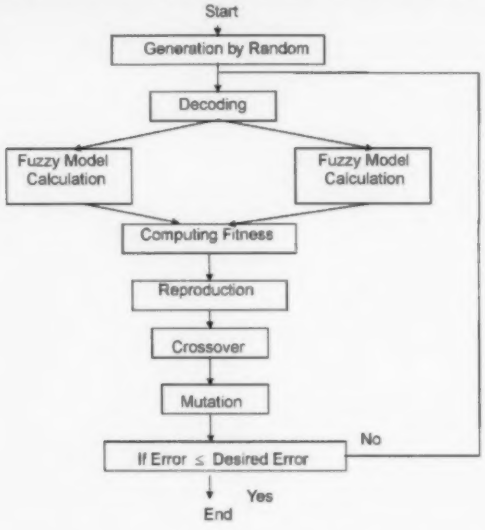
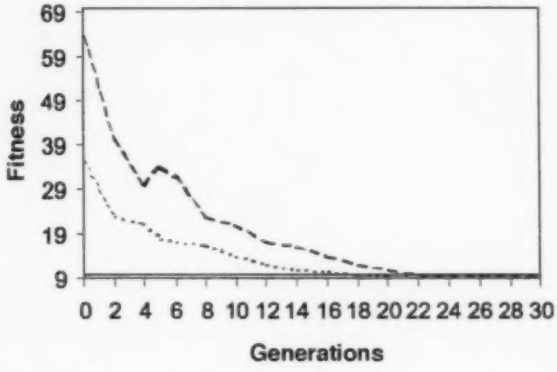


Fig. 4. Changes of fitness versus generation (or iteration) in the genetic algorithm learning process for fitting the WCVI Pacific herring data. A similar figure can be produced for the SEAK pink salmon data. The short-dashed, long-dashed, and solid lines from the top to the bottom represent the worst, median, and best fitness values in the population in each generation.



sequent generations (the term "generation" used in genetic algorithm terminology is equivalent to an "iteration" in other optimization methods). In this case, the uppermost line shows the worst fitness values in the population, the lowest line shows the best fitness values, and the middle line shows the median fitness value in the population. In this example, it takes about 20 generations or learning cycles to find the optimal solution. The genetic learning algorithm is written in Turbo C++. The two input variables and one output variable are standardized to lie in the interval [0,1].

Data sources and results

Two stock–recruitment data sets were analyzed. The first example, taken from Quinn and Deriso (1999), is for SEAK pink salmon from 1960 to 1989. The second example is for the WCVI Pacific herring stock (Chen and Ware 1999). To illustrate the capabilities of the Fuzzy–GA model to reconstruct historical stock–recruitment time series and to forecast future recruits, the analysis is divided into two parts. The first part uses the complete time series to test the ability of the Fuzzy–GA model to reconstruct the historical pink salmon and Pacific herring recruitment time series. In the second part, we reserved 5 years of data (with largest range of variation in recruitment during this period) from the fitting process to independently test the ability of the Fuzzy–GA model to forecast future recruits. To facilitate model comparison, the data in both parts were analyzed using the simple Ricker climatic stock–recruitment model, a neural network model, and the Fuzzy–GA model.

SEAK pink salmon

Data description and preliminary analysis

Detailed data descriptions, analyses, and the stock–recruitment time series for SEAK pink salmon from 1960 to 1989 were taken from Quinn and Deriso (1999). The right-hand panels in Fig. 5 reproduce the recruitment time series. To determine the stock–recruitment relationship, Quinn and Deriso (1999) explored eight models, which included the constant model, density-independent model, Bevertion–Holt model, Ricker model, Cushing model, Deriso–Schnute model, Shepherd model, and gamma model. Table 3.2 in Quinn and Deriso (1999) summarized the fitted results for these nine models. In brief, the density-independent model fit the data better than the constant model and had a statistically significant coefficient, which implies that recruitment tends to increase with spawner biomass. For the six density-dependent stock–recruitment models, the estimated density-dependent parameter was not significantly different from zero, and the coefficient of determination (R^2) was only 20–25%.

To try to account for some of the unexplained variation in recruitment, Quinn and Deriso (1999) introduced an environmental factor, average annual SST off Sitka, Alaska, into the analysis. They found that a Ricker stock–recruitment model, which included SST, produced a statistically significant fit to the recruitment time series: the RMSE was 4729.5 and the correlation coefficient (R) was 0.67.

Fuzzy–GA model analysis

To perform the Fuzzy–GA model analysis, the data were rescaled from 0 to 1. For the fuzzy membership function in Fig. 1, k_1 and k_2 for the spawner and SST input variables were chosen to be the mean values (0.25 and 0.5 for the rescaled data) to reflect the fuzzy membership function for fuzzy set "middle." As shown in Fig. 1, the spawner and SST inputs are classified into three categories ("small," "middle," and "high" for spawner input and "low," "middle," and "high" for SST input). This classification leads to 27 fuzzy parameters: a_i, b_i, and c_i (i = 1 to 9) in the nine fuzzy rules. These parameters are estimated from genetic algorithm operations to minimize the RMSE (Table 2). The resulting RMSE of the observed and fitted pink salmon recruits is 3838.5 (units in thousands of pink salmon) and R is 0.79. The top panel plots in Fig. 5 illustrate the Fuzzy–GA

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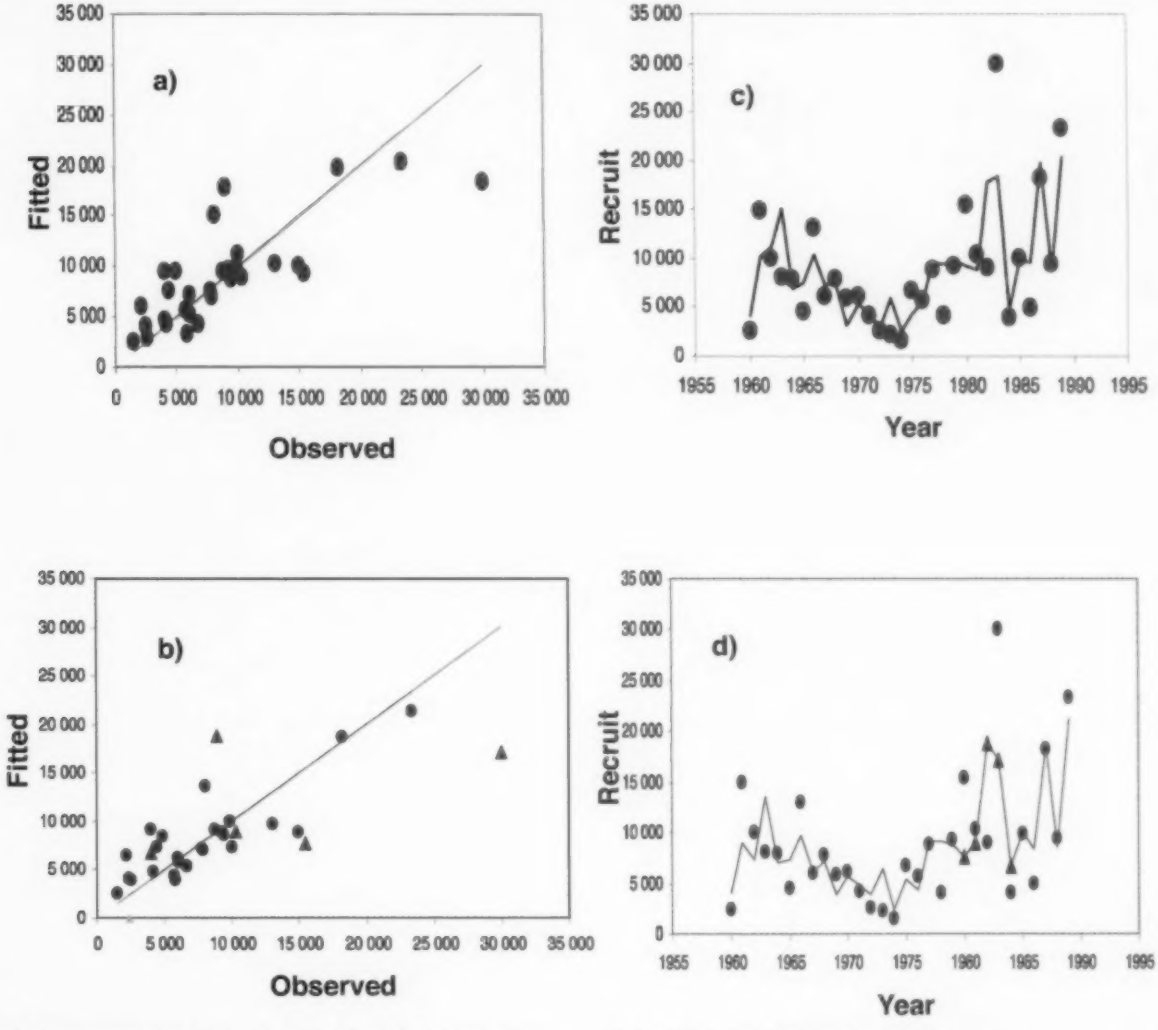
Fig. 5. SEAK pink salmon recruitment data. (*a* and *c*) The results using all of the data. (*a*) The relationship between the observed and the Fuzzy–GA modelled recruits with the 1:1 line. (*c*) The recruitment time series, where the line indicates the modelled recruitment estimates. (*b* and *d*) The results for the forecasting analysis. The points indicated by triangles were not included in the fitting process for forecasting. (*b*) The relationship between the observed and the Fuzzy–GA modelled recruits with the 1:1 line. (*d*) The reconstructed recruitment time series. Circles indicate the observed recruitment data points and the five triangles represent the Fuzzy–GA model forecasts.



model reconstruction of the historical SEAK pink salmon recruitment time series.

A 2–2–1 neural network model was also applied to these data (Fig. 6). A detailed description of the model can be found in Chen and Ware (1999). This model produced an RMSE of 4351.3 and R of 0.72. Model fits using one and three hidden neuron models (i.e., a 2–1–1 neural network and a 2–3–1 neural network) produced similar results.

The ability of the Ricker climatic, neural network, and Fuzzy–GA models to fit the pink salmon data is summarized in Table 3. The Fuzzy–GA model produced the best fit based on the RMSE and the value of R between the observed and modelled recruitment. The neural network model performed better than the Ricker climatic model.

Pink salmon recruitment forecasting

Data from 1980 to 1984 were reserved for forecasting because there was a large range in recruitment during this period. The Fuzzy–GA, neural network, and the Ricker climatic models were fit to the rest of the data to train the model. The performance of these models in fitting the data is summarized in Table 3. For the training process, both the Fuzzy–GA and neural network models outperformed the Ricker climatic model.

The resulting models were then used to forecast recruitment for the 1980–1984 period. In this case, the RMSE was used as the performance criterion because the correlation is not a reliable criterion based on only 5 years of data. Table 3 indicates that the Fuzzy–GA model produced a better fore-

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Table 2. Optimal fuzzy logic model parameters obtained from the genetic learning algorithm.

	SEAK pink salmon			WCVI Pacific herring		
Rule	*a*	*b*	*c*	*a*	*b*	*c*
1	0.73	1.18	–0.08	–1.02	1.37	–1.15
2	0.26	–0.22	0.27	–0.03	–0.83	0.75
3	–1.63	0.7	–0.04	0.15	0.76	–0.24
4	0.41	0.34	0.03	–0.13	0.07	0.8
5	0.07	0.82	0.04	–0.3	–0.99	0.77
6	0.31	0.3	–0.02	0.04	0.93	–0.52
7	0.03	–0.76	1.24	0.76	1.44	0.82
8	0.5	–0.16	0.02	–1.07	0.8	0.5
9	1.57	1.69	1.85	–1	0.03	0.45

cast than the neural network model, which in turn was better than the Ricker climatic model.

WCVI Pacific herring

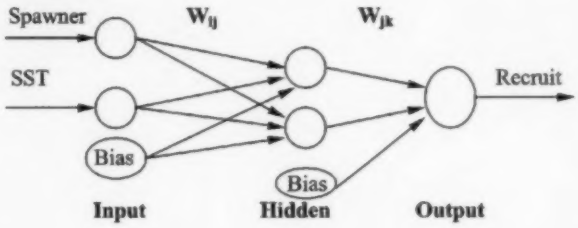
Data description

A long-term research program on the WCVI Pacific herring stock indicates that the key variables that affect the biomass of 3-year-old Pacific herring recruits (in year t) are the biomass of spawners (i.e., parents) in the year in which the recruits were born ($t - 3$ years), the annual mean SST in year $t - 3$, and the biomass of Pacific hake (*Merluccius productus*) (an important predator) in year $t - 3$ (Ware 1991; Ware and McFarlane 1995; Chen and Ware 1999). Temperature is believed to be a proxy "signal" that reflects interannual variability in the relative biomass of larval and juvenile Pacific herring predators and possibly some important components of the Pacific herring food supply. In general, cooler (warmer) temperatures tend to produce larger (smaller) recruitments 3 years later. This negative correlation between the annual SST and the biomass of WCVI Pacific herring recruits has been a consistent feature of the recruitment time series since Tester (1948) discovered it about 50 years ago. Recent work indicates that the relationship between parent spawners and recruits is masked by the temperature effect. The underlying dome-shaped spawner–recruit relationship becomes apparent if the recruitment data are sorted into two groups: year-classes born in years of above-average temperature and year-classes born in years of below-average temperature. For each group, there is a significant Ricker-like relationship between spawner and recruit biomass (Ware 1996). To be consistent with the SEAK pink salmon example, we examined the importance of the Pacific herring spawning biomass and the annual SST for recruitment using the Fuzzy–GA model.

Fuzzy–GA model analysis

To implement the model, the input data were rescaled from 0 to 1. Values for k_1 (for spawner biomass) and k_2 (for SST) in the fuzzy membership function illustrated in Fig. 1 were chosen to be the mean values (0.22 for spawner biomass and 0.5 for SST) corresponding to the rescaled data to reflect the fuzzy membership function for fuzzy set "middle." Twenty-seven fuzzy parameters, a_i, b_i, and c_i based on nine fuzzy rules, were estimated by the genetic algorithm and are summarized in Table 3. Figure 4 illustrates the performance of the genetic algorithm for different generations. The final result produced an RMSE between the observed and fitted WCVI Pacific herring recruits of 9.98 and a linear R of 0.72. For comparison, a Ricker climatic model was also fit to the data, with a resulting RMSE of 12.77 and an R of 0.53. We also examined the fit of the data to a 2–1–1, 2–2–1 (Fig. 6), and 2–3–1 neural network model (see Chen and Ware 1999 for details). Similar RMSE and R values were obtained. For example, the RMSE from the 2–2–1 neural network model was 10.22 and R was 0.70.

Fig. 6. Topology of a two-input, two-hidden, and one-output (2–2–1 in neural network terminology) neural network model. The two inputs are spawner biomass and SST. The output is the resulting biomass of recruits. W_{ij} and W_{jk} are weights, which represent the link between the inputs and the output. The weights contain all the information about the network. Therefore, the objective is to train the network to find a series of weights that yield an output signal that has a small error relative to the observed output.



A comparison of the results produced by the three different models is summarized in Table 3. The Fuzzy–GA model produced the best fit to the original recruitment time series based on the estimated RMSE and the R values (Figs. 7*a* and 7*c*). The neural network model performed better than the Ricker climatic model, which is consistent with the results obtained by Chen and Ware (1999).

Pacific herring recruitment forecasting

To compare the forecasting abilities of the three models, we created a new time series where the data for the years 1972–1976 were omitted. This 5-year period was selected because it contained the largest contrast in recruitment (Fig. 7). The Fuzzy–GA, neural network, and Ricker climatic models were fit to the remaining data. The RMSE and the R between the observed and estimated recruits are shown in Table 3. In terms of performance, we obtained the same results with the shorter time series as we did with the complete time series: the Fuzzy–GA model produced the best results followed by the neural network model. The Ricker climatic model produced the poorest fit.

The three models were then used to forecast the abundance of recruits in the 1972–1976 year-classes. In this case, the RMSE was used as the performance criterion because with only 5 years of data, the correlation is not a reliable criterion. The forecasting results for the three models are summarized in Table 3. Once again, the Fuzzy–GA model yielded the best results.

In summary, the descriptive and forecasting capabilities of a Takagi–Sugeno type fuzzy logic model with a genetic learning algorithm are evaluated for two independent sets of fish stock–recruitment. The model is capable of incorporat-

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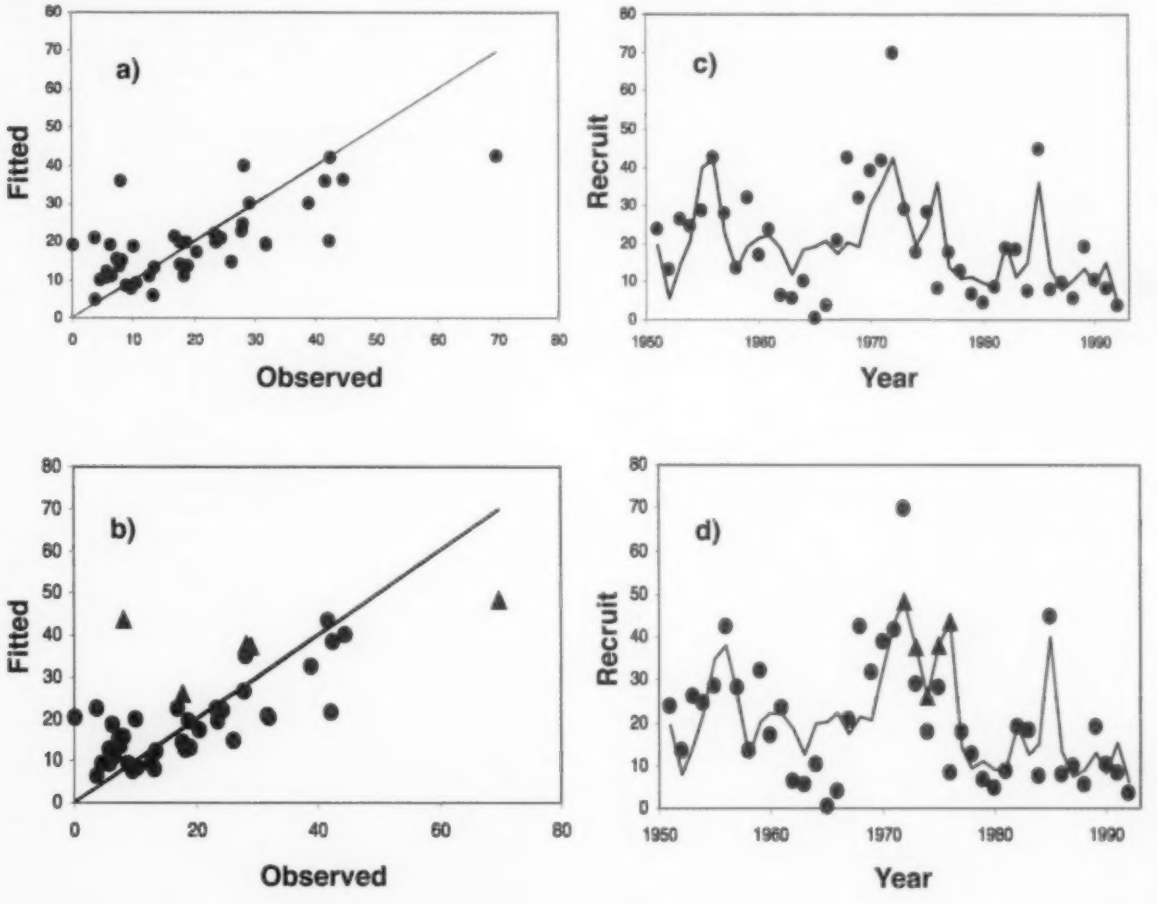
Table 3. Fitting and forecasting summary information for the Ricker climate stock–recruitment, neural network, and Fuzzy–GA models.

		SEAK pink salmon		WCVI Pacific herring	
	Model	R	RMSE	R	RMSE
All data	Ricker	0.6729	4729.46	0.5291	12.77
	Neural network	0.7191	4351.25	0.7042	10.22
	Fuzzy–GA	0.7899	3838.54	0.7201	9.98
Five years of data reserved					
Fitting	Ricker	0.7640	3357.16	0.4580	11.85
	Neural network	0.7882	3113.09	0.6139	9.88
	Fuzzy–GA	0.8556	2613.82	0.7657	8.06
Forecast	Ricker	0.3722	9111.13	0.5833	26.15
	Neural network	0.4794	8368.87	0.3474	21.69
	Fuzzy–GA	0.4641	8167.48	0.5431	19.82

Fig. 7. WCVI Pacific herring recruitment data. (*a* and *c*) The results using all of the data. (*a*) The relationship between the observed and the Fuzzy–GA modelled recruits with the 1:1 line. (*c*) The reconstructed recruitment time series, where the line indicates the modelled recruitment estimates. (*b* and *d*) The results for the forecasting analysis. The points indicated by the triangles were not included in the fitting process for forecasting. (*b*) The relationship between the observed and the Fuzzy–GA modelled recruits with the 1:1 line. (*d*) The reconstructed recruitment time series. Circles indicate the observed recruitment data points and the five triangles represent the Fuzzy–GA model forecasts.



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ing the effects of environmental interventions on the stock–recruitment process. Fuzzy logic operations are used to categorize the input–output information on spawner biomass, recruitment, and environmental interventions into fuzzy sets with an associated degree of membership function. The inherent uncertainties in the data were taken into account by the fuzzification process. The Fuzzy–GA model is capable of empirically approximating the underlying nonlinear stock–recruitment relationship and can also provide a crisp and simple functional relationship among the inputs and outputs according to the fuzzy rules (nine in this application). An important feature of the Fuzzy–GA model is that the functional relationships described by the fuzzy rules can be chosen to more realistically describe the biological processes that affect recruitment.

The application of the genetic algorithm surpasses any conventional mathematical search algorithm because the algorithm used here utilized 240 simultaneous starting points in the searching space. Consequently, the genetic algorithm has a much lower chance of converging to some local optima, especially when several parameters are involved in the optimization. Accordingly, we believe that a fuzzy logic stock–recruitment model with a genetic optimization algorithm can be a useful tool for forecasting recruitment to fish population.

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Appendix. An example of the fuzzy reasoning process

To illustrate the fuzzy reasoning process, we take the data from year 1951 WCVI Pacific herring stock. The corresponding values for Pacific herring recruitment, spawner biomass, and SST after standardization are 0.34, 0.17, and 0.35, respectively. Using the spawner biomass and SST input values, the modelled values y_i from Rules 1–9 can be calculated from Step 1 with the parameters from Table 2, which are $(y_1, y_2, y_3, y_4, y_5, y_6, y_7, y_8, y_9) = (-0.84, 0.45, 0.05, 0.81,$

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0.37, –0.18, 1.45, 0.60, 0.30). From the fuzzy membership function defined in Fig. 1, the weight vector α corresponding to Rules 1–9 can be inferred from the min operation (^) in Step 2: $\alpha = (\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6, \alpha_7, \alpha_8, \alpha_9) = (0.23, 0.23, 0, 0.3, 0.7, 0, 0, 0, 0)$. Therefore, the output value from the fuzzy logic model can be obtained from eq. 1 as $y = 0.28$. The model error for the year is then $0.34 - 0.28 = 0.06$. The fitness function (i.e., the objective function) is therefore the sum of squares of the errors.

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Cause of pH decline in stream water during spring melt runoff in northern Sweden

Hjalmar Laudon, Olle Westling, and Kevin Bishop

Abstract: This study has sought to distinguish the anthropogenic and natural factors that drive episodic pH decline in northern Sweden. Approximately 600 stream water chemistry samples from 12 streams during the spring melt runoff of 1997 and 1998 were collected. Although the acid deposition levels of the region are relatively low (2–4 kg SO_4^{2-}-S·ha^{-1}·year^{-1}), the pH decline in all of the almost two dozen spring melt events ranged from nearly 1 to 3 pH units. By using the sum of base cation concentration as a dilution index and an organic acid pH model, the sources contributing to the pH decrease were quantified. For a majority of the spring melt events, organic acids contributed over 75% of the acidity at peak runoff (minimum pH). In only three of the monitored events was the anthropogenic SO_4^{2-} contribution as high as that from natural sources. NO_3^- did not contribute to the pH decline during spring melt in this study. An interannual variation was observed that was probably due to a larger anthropogenic deposition load during the winter of 1997–1998 and a more rapid snowmelt during the spring of 1998.

Résumé : Notre étude a tenté de faire la part entre les facteurs anthropiques et naturels qui régissent la baisse épisodique du pH dans le nord de la Suède. Nous avons recueilli environ 600 échantillons d'eau dans douze cours d'eau pendant la fonte des neiges au printemps de 1997 et 1998. Bien que les retombées acides dans la région soient relativement faibles (2–4 kg SO_4^{2-}-S·ha^{-1}·an^{-1}), la baisse du pH observée à chaque fois dans près de deux douzaines d'événements de fonte printanière allait de près d'une à trois unités de pH. En prenant comme indice de dilution la somme de la concentration des cations basiques, et en utilisant un modèle du pH des acides organiques, nous avons quantifié les sources contribuant à la baisse du pH. Pour la majorité des événements de fonte printanière, les acides organiques représentaient plus de 75% de l'acidité au pic d'écoulement (pH minimum). Dans trois seulement des événements étudiés, l'apport anthropique de sulfate était aussi haut que celui des sources naturelles. Le nitrate ne contribuait pas à la baisse du pH pendant la fonte printanière au cours de notre étude. Nous avons observé une variation interannuelle qui était probablement due à une charge anthropique plus forte pendant l'hiver 1997–1998 et à une fonte plus rapide au printemps 1998.

[Traduit par la Rédaction]

Introduction

Episodic acidification during snow and rain events has been well documented in North America and Europe during the last decades (Davies et al. 1992; Wigington et al. 1992). The change in Al speciation accompanying large pH depressions has been shown to cause stress and mortality in many aquatic species (Carline et al. 1992; Baker et al. 1996). The general conclusion has been that episodic acidification can result from a combination of both natural and anthropogenic processes. In a few cases, the potential for natural pH decline by dilution of acid neutralization capacity (ANC) (Molot et al. 1989), organic acidity (Campbell et al. 1992), and SO_4^{2-} derived from sediments or bedrock (Jansson and Ivarsson 1994) has been acknowledged. Since most previous episode studies have concentrated on areas of relatively high atmospheric depositions rates, these natural influences have often been deemed less important than the anthropogenic SO_4^{2-} and NO_3^- contribution to the pH decline.

Although acid deposition during episodes has been found to be a major threat to aquatic ecosystems in many areas of the world, it is only in Scandinavia that large-scale remediation is attempted. During the last decade, the Swedish government has subsidized liming with approximately 500 million Swedish crowns (>$75 million (US)) to protect and restore surface waters in northern Sweden from anthropogenic acidification during spring melt events. There is, however, considerable concern that liming is not appropriate in water with high total organic C (TOC) levels where natural acidity is, at least partly, responsible for the low pH and buffering capacity (Bishop 1991; Kullberg et al. 1993; Warfvinge et al. 1995).

The purpose of this study was to better understand and quantify anthropogenic and natural driving factors of episodic acidity in northern Sweden to improve the understanding of the future liming needs in the region. Northern

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Sweden is a region with a relatively low anthropogenic acid deposition rate (2–4 kg SO_4^{2-}-S·ha^{-1}·year^{-1}) but where streams commonly have low pH values (<5.0) during runoff episodes, especially spring melt events (Laudon 1999). Previous episode studies in the area have shown that anthropogenic acidification (Jacks et al. 1986; Ahlström 1994) as well as natural processes (Bishop 1991; Jansson and Ivarsson 1994; Laudon and Bishop 1999) can be the main contributing factor to the pH decline during episodes. These isolated examples of natural and anthropogenic pH depressions during episodes of high flow, however, are not sufficient to reliably assess the cause and extent of episodic acidity in northern Sweden. The work presented here will compare the similarities and differences in the stream water chemistry during the 1997 and 1998 spring melt events of 12 geographically representative streams in northern Sweden. As part of this work, the project sought to model the influence of TOC on the pH of organic-rich streams during spring melt, since this is a prerequisite for distinguishing and quantifying natural from anthropogenic acidity. The paper will also provide an overview of the project and the methodologies used and will summarize the characteristics of the study sites, including anthropogenic acid deposition.

Methodology

Field locations

Selection of the 12 study streams in northern Sweden (Fig. 1) was based on six criteria: (*i*) two east to west transects (one more southerly and one more northerly), where two streams in each transect were located in the coastal area, two in the inland area, and two in the mountain range along the western border to Norway, (*ii*) all catchment areas should be smaller than 65 km^2 but large enough to sustain an indigenous fish population, (*iii*) no lakes should be present in the main streams, (*iv*) no liming activity should have been carried out within the drainage areas, (*v*) previous stream water chemistry data were required from each stream (at least from baseflow), and (*vi*) proximity to plowed roads was also considered in order to facilitate spring episode sampling.

The northern transect is located entirely within Västerbotten County whereas the southern transect traverses the counties of Dalarna, Gävleborg, and Västernorrland (Fig. 1). The characteristics of the catchments are presented in Table 1. With the exception of the mountain catchments, the forested areas are mainly covered by Scots pine (*Pinus sylvestris*) in the upper, drier areas and Norway spruce (*Picea abies*) in the lower terrain. The forests in the mountain sites consist mainly of mountain birch (*Betula tortuosa*).

Sampling

Water sampling was carried out by local observers collecting grab samples and recording stage height. A stage–discharge relationship was established for five of the 12 streams using hand-held current meters or a semiautomatic salt dilution technique. The sampling program was based on weekly samples of baseflow prior to the onset of episodes and then daily sampling until the water level returned close to a baseflow level. All samples were collected in acid-washed 250-mL polyethylene bottles. The samples were stored cold and in the dark until sent by mail to the laboratory on the first possible weekday.

Laboratory analysis

All samples collected in this study were analysed for pH and absorbance (about 600 samples in total). Since the partial pressure of CO_2 (pCO_2) will influence pH above 5.0, both the ambient pH and aerated pH of all samples were measured in the laboratory at 25°C. The aeration of the pH samples was carried out for 20 min with compressed standard air (pCO_2 = 380 ppm).

All samples were measured twice for absorbance at 254 nm. TOC was measured in about 30% of the samples with a Shimadzu DOC-500 using catalytic combustion. The absorbance for each stream was then calibrated separately against TOC analyses. The standard deviations for the calibrations were between 0.35 and 0.80 mg·L^{-1} for the different subsets. About half of all samples collected were analysed for inorganic anions and cations. The samples from the northern transect and Kälsbäcken were analysed at the Geological Survey, Prague, Czech Republic, using liquid ion chromatography for inorganic anions and atomic absorption spectrophotometry for cations. The sites in the southern transect were analysed for SO_4^{2-} and Cl^- using liquid ion chromatography at the Department of Environmental Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. NO_3^- was analysed using flow injection analysis, while base cations (BC) (Ca^{2+}, Mg^{2+}, K^+, and Na^+) were analysed using atomic absorption spectrometry at MEANA, Uppsala, Sweden. Subsamples for cations were preserved with suprapure nitric acid before analysis, while subsamples for anions had been frozen after collection. The analytical precision for major anions and cations was ±5% and for Si was ±7%.

Aluminum speciation was analysed using a flow injection analysis technique (Clarke et al. 1992) at the Department of Forest Ecology, SLU, Umeå, Sweden. Total and labile monomeric Al was measured for the northern transect and Kälsbäcken.

National atmospheric deposition monitoring data from the Swedish Environmental Research Institute (IVL) was used to assess the acid deposition levels for the winters of 1997 and 1998 as well as to compare this study period with atmospheric deposition levels from previous years (Hallgren Larsson et al. 1995; Kindbom et al. 2000).

Acid–base modeling

The buffering capacity or ANC is a measure of natural water's capacity to consume H^+. ANC is commonly calculated as the difference between the sum of BC and strong mineral acids:

$$(1) \quad ANC = [BC] - 2[SO_4^{2-}] - [Cl^-] - [NO_3^-].$$

ANC can also be calculated as

$$(2) \quad ANC = [HCO_3^-] + 2[CO_3^{2-}] + [RCOO^-] + [OH^-] - [H^+] - n[Al^{n+}]$$

where $RCOO^-$ are the dissociated organic acid anions and Al^{n+} denotes positively charged inorganic monomeric Al species. Previous studies have shown that the inclusion of the monomeric labile Al fraction and Fe does not significantly improve the agreement between measured and model-predicted pH at the pH range of these stream water samples. Therefore, Al^{n+} can be excluded from the calculations (Driscoll et al. 1994). Hence, by combining eqs. 1 and 2 (while excluding Al^{n+}), the H^+ concentration of surface water can be calculated as

$$(3) \quad [H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [RCOO^-] + [OH^-] - [BC] + 2[SO_4^{2-}] + [Cl^-] + [NO_3^-].$$

The concentrations of HCO_3^- and CO_3^{2-} depend on both the pCO_2 and the pH of the sample. In the pH range of this study, the CO_3^{2-} concentration can be excluded from the calculation. The concentration of HCO_3^- was calculated using

$$(4) \quad [HCO_3^-] = (pCO_2/[H^+])K_{eq}$$

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Fig. 1. Monitoring sites for precipitation chemistry and stream water chemistry in northern Sweden.

For the dissociated organic acid anions $RCOO^-$, a number of modeling approaches have been presented in the literature, including continuous pK_a distribution models and discrete, multi-pK_a models (Driscoll et al. 1994) as well as a single but pH-dependent pK_a model (Oliver et al. 1983). According to the model of Oliver et al. (1983), the acid dissociation constant (pK_a) for TOC can be calculated as a second-order function of pH (eq. 5) (TOC is used instead of dissolved organic C (DOC) in the optimization procedure, since DOC and TOC differ by less than 5% during spring melt events (H. Laudon, unpublished data)):

$$(5) \qquad pK_a^{\text{optimized}} = a + b \times \text{pH} + c \times \text{pH}^2$$

The three constants in eq. 5 and the site density of TOC (originally set to 10 μequiv.·mg DOC^{-1} by Oliver et al. (1983)) were cali-

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Table 1. Description of stream chemistry monitoring sites.

Northern transect						
	Mountains		Inland		Coast	
	Rukkebäcken	Stopelebäcken	Sörbäcken	Mellansjöbäcken	Stridbäcken	Lillån
Drainage area (km^2)	20	60	62	26	9	21
Wetland area (%)	<1	13	14	32	40	23
Lake area (%)	<1	2	<1	3	<1	<1
Forest area (%)	5	60	86	65	30	70
Bare rock/scrub (%)	95	25	<1	<1	30	7
Main geology	Mica schist	Cambrosilurian sedimentary rock	Porphyritic Revsund granite	Porphyritic Revsund granite	Migmatite, veined gneiss	Migmatite, veined gneiss
Main soils	Till	Till	Till	Till	Till	Sediment/till
Elevation (m asl)	550–1407	480–1032	290–560	330–570	90–220	10–185
Stream order	Second	Second	Third	Second	First	Second
Southern transect						
	Mountains		Inland		Coast	
	Upper Göljeån	Lower Göljeån	Nyckelmyrbäcken	Acktjärnsbäcken	Sörjabäcken	Kälsbäcken
Drainage area (km^2)	6.5	20.8	7.5	6.5	17	4.3
Wetland area (%)	<1	2	5	54	17	18
Lake area (%)	<1	<1	<1	<1	<1	1
Forest area (%)	<1	33	95	46	83	74
Bare rock/scrub(%)	99	65	<1	<1	<1	7
Main geology	Sandstone	Sandstone	Granite/pegmatite	Sandstone	Metavolcanic rock	Granite
Main soils	Till	Till	Till	Till	Till	Till
Elevation (m asl)	867–984	464–984	203–402	433–508	190–350	55–290
Stream order	Second	Third	Third	Second	Third	Second

brated in this study. This was accomplished by calculating an effective pK_a value for each individual water sample:

$$(6) \qquad pK_a^{effective} = [H^+][RCOO^-]/[RCOOH]$$

where $RCOO^-$ was calculated from the charge balance of each sample (all positive ions minus all negative inorganic ions, including HCO_3^-). RCOOH is the protonized form of organic acids. The site density and the three constants in eq. 5 were then calibrated by minimizing the sum of squared errors between the optimized pK_a (eq. 5) and the effective pK_a (eq. 6).

Contribution of acidity

To evaluate the relative importance of natural and anthropogenic acidity during the spring melt, the concentrations of SO_4^{2-}, NO_3^-, and $RCOO^-$ were normalized to the dilution of a dilution tracer (Campbell et al. 1992; Laudon et al. 1999). The dilution index (DI) at any time (t) during the spring melt episode was calculated as the ratio between the tracer concentration of a specific sample during the episode and an average baseflow concentration preceding the spring melt in each site:

$$(7) \qquad DI(t) = [tracer(t)]/[tracer_{baseflow}].$$

The normalized contribution of free acidity (protons) during the spring melt was calculated by comparing the dilution of the individual acid anions with the dilution index, in this case, BC. Therefore, by normalizing the variation of the different acid anions with the BC variation during the spring runoff, it is possible to estimate the relative importance of SO_4^{2-}, NO_3^-, and $RCOO^-$ for the declining buffer capacity and pH at any point in time during the episode. Cl^- does not contribute with acidity, since it originates from marine sources, deposited with precipitation as a neutral salt. The anthropogenic (SO_4^{2-} and NO_3^-) and the natural (organic acid) sources of protons (free acidity) during the spring melt events were calculated as

$$(8a) \qquad H^+_{SO_4^{2-}} = SO_4^{2-}(t) - SO_4^{2-}{}_{baseflow}DI(t)$$

$$(8b) \qquad H^+_{NO_3^-} = NO_3^-(t) - NO_3^-{}_{baseflow}DI(t)$$

$$(8c) \qquad H^+_{RCOO^-} = RCOO^-(t) - RCOO^-{}_{baseflow}DI(t)$$

Results

Deposition

The deposition of S during the mid-1990s was less than 3 kg $S{\cdot}ha^{-1}{\cdot}year^{-1}$ in open fields and less than 5 kg $S{\cdot}ha^{-1}{\cdot}year^{-1}$ in the forests of northern Sweden. Deposition of NO_3^--N during this period was normally less than 2 kg $N{\cdot}ha^{-1}{\cdot}year^{-1}$. The acid contribution from the deposition of S and N was in the range 0.02–0.16 kg $H^+{\cdot}ha^{-1}$ during the winters of 1996–1997 and 1997–1998 (Hallgren Larsson 1999).

There is a marked deposition gradient from the east coast to the inland western parts of northern Sweden, with the highest deposition in the coastal area (Fig. 2). The winter of 1996–1997 had a lower deposition of strong acids (especially from S) compared with several preceding winters, despite normal precipitation amounts during the winter of 1996–1997. Open-field S deposition in the coastal and southern part of the study area during December–April was less than 1 kg $S{\cdot}ha^{-1}$, with a few exceptions. In the inner part of northern Sweden, S deposition was less than 0.5 kg $S{\cdot}ha^{-1}$.

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Fig. 2. Sulfur deposition (kg·ha^{-1}) in northern Sweden during the winter period (December–April) (Hallgren Larsson 1999).

During the winter of 1997–1998, S deposition was between 1.2 and 2 kg S·ha^{-1} in the coastal and southern part of the study area. The inland areas and northern areas had deposition between 0.3 and 1.2 kg S·ha^{-1} (Fig. 2).

Earlier results from the national precipitation network during the same months indicate that the bulk wet deposition of S was 1.5–4 times higher during the winters of 1987–1989 compared with this study but with a large variation between years (due partly to a large variation in precipitation amounts (Kindbom et al. 2000)). This decade-long decline is a result of declines in European S emissions (Lövblad et al. 1995).

The variation in NO_3^--N deposition, normally less than 1 kg N·ha^{-1} during the winter period at most sites, was relatively small between 1991 and 1998. The NO_3^--N deposition during the winter of 1997–1998 in the coastal and southern part of northern Sweden exceeded 1 kg N·ha^{-1}, which was about 50% higher than during the winter of 1996–1997 (Hallgren Larsson 1999).

Hydrology

In the northern transect, the 1997 spring melt started in early May and reached its peak flow several weeks later. In the southern transect, snow started melting at most sites in early March but ceased later due to colder weather. The 1998 spring melt runoff was preceded by cold weather (–20°C) in large areas of northern Sweden for almost a month. At the end of April, the region was hit by very warm weather, which initiated a rapid runoff event and flooding of many small streams along the coast and inland of northern Sweden. At most sites, with Nyckelmyrbäcken being an exception, the sampling period commenced before the spring melt runoff started.

Baseflow conditions

There was a large spatial variation in the baseflow chemistry between the different study sites, ranging from well-buffered, low-TOC streams with an aerated pH above 7.5 to Stridbäcken with low buffering capacity, moderately high TOC, and a baseflow pH below 5.5.

In general, the highest baseflow TOC levels, 8–16 mg·L^{-1}, were found in the inland and along the coast of the Baltic Sea. The low TOC concentrations were mainly in the mountain sites along Sweden's western border with Norway (Fig. 3*a*). The highest buffering capacities in the northern transect were in the mountain sites, whereas the lowest ANC

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Fig. 3. Variation in (*a*) TOC, (*b*) ANC, (*c*) SO_4^{2-}, and (*d*) BC between baseflow and peak flow (minimum pH) during the 1997 (first bar) and 1998 (second bar) spring melt events in the study streams. Gray bars denote a decreasing concentration with increasing discharge. Hatched bars depict an increasing concentration with a rising runoff.

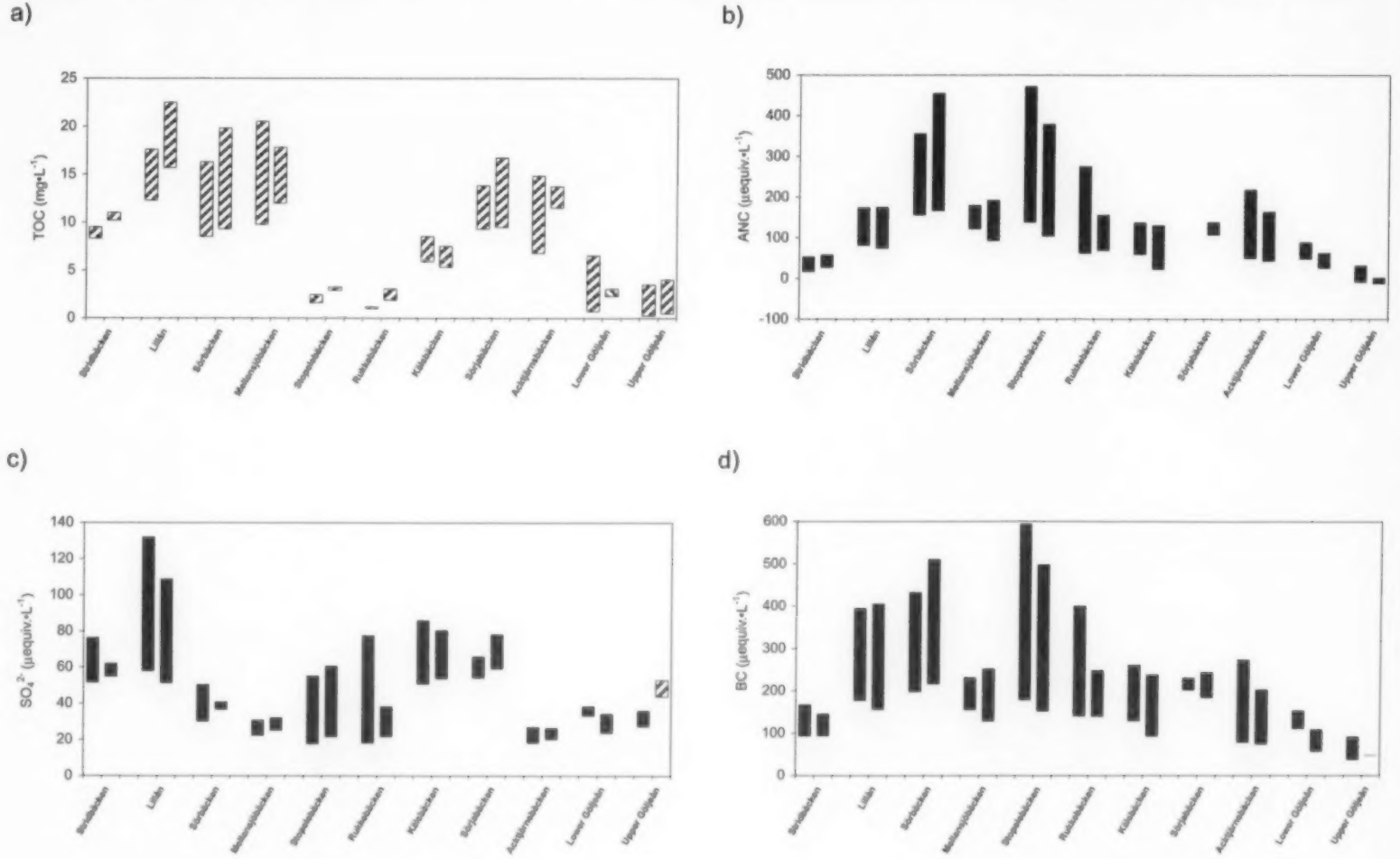

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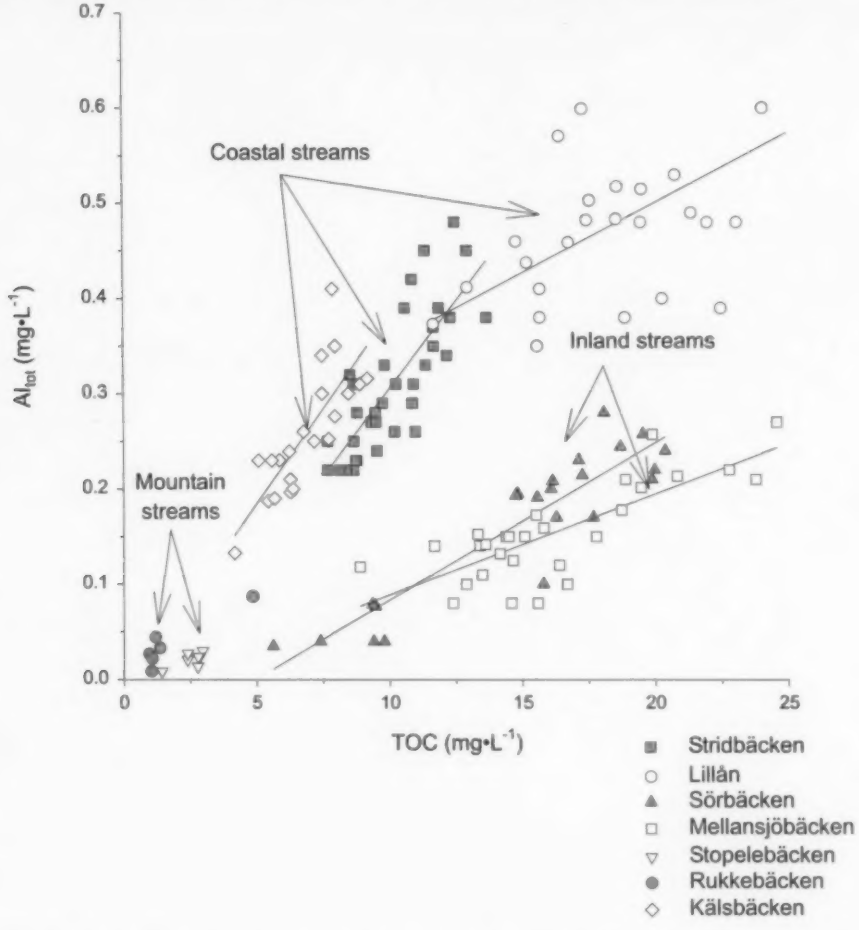
Fig. 4. Relationship between TOC and total Al for the streams in the northern transect, including Kälsbäcken.



sites are located on the coast (Fig. 3*b*). In the southern transect, the pattern was the opposite, with the most highly buffered base flow streams at the coastal sites.

The highest levels of baseflow SO_4^{2-} were along the coast, which corresponds to the deposition pattern of SO_4^{2-} (Fig. 2). However, these sites also lie below the highest postglacial marine coastline where the presence of S-rich soils of marine origin can cause high SO_4^{2-} levels in the baseflow. Within the Lillån catchment, where deposits of these S-rich sediments are well known, this is reflected in the highest baseflow S values (132–95 μequiv.·L^{-1}) of all the study sites. In the southern transect, the streams with catchment outlets below the highest coastline, all have a baseflow concentration of SO_4^{2-} above 70 μequiv.·L^{-1}, whereas the streams with outlets above the highest coastline have a concentration of less than 40 μequiv.·L^{-1}. The pattern is similar, but less pronounced, in the northern transect. Bedrock along the coast also has a higher S content than the inland and mountain region (Jansson and Ivarsson 1994). Oxidation and subsequent release of SO_4^{2-} from these soils or till originating from S-rich bedrock in response to seasonal fluctuations of the groundwater level are thus potential sources of natural SO_4^{2-} acidity in the coastal area (Jansson and Ivarsson 1994).

Episodic response during spring melt runoff

During the observed spring melt events, a negative relationship between stream flow and most chemical parameters was found for all streams. Both pH (aerated and ambient) and ANC declined as soon as the spring melt runoff reached the streams. Except for the 1997 spring melt at Stridbäcken, the most acid conditions occurred close to the maximum flow. Stridbäcken experienced its minimum pH during a smaller flow peak during the end of the melt season. Only the Upper Göljeån site experienced negative ANC values during the peak runoff (Fig. 3*b*).

A similar pattern of concentration declines with increasing flow was observed for most mineral elements, including SO_4^{2-} (Fig. 3*c*) and BC (Fig. 3*d*). TOC and Al, however, displayed a generally positive relationship with the increase in discharge. At the onset of spring melt, TOC increased in concentration from that of baseflow and reached its maximum level (up to 30 mg·L^{-1} in some inland and coastal streams) a few days prior to peak flow. For most streams, the

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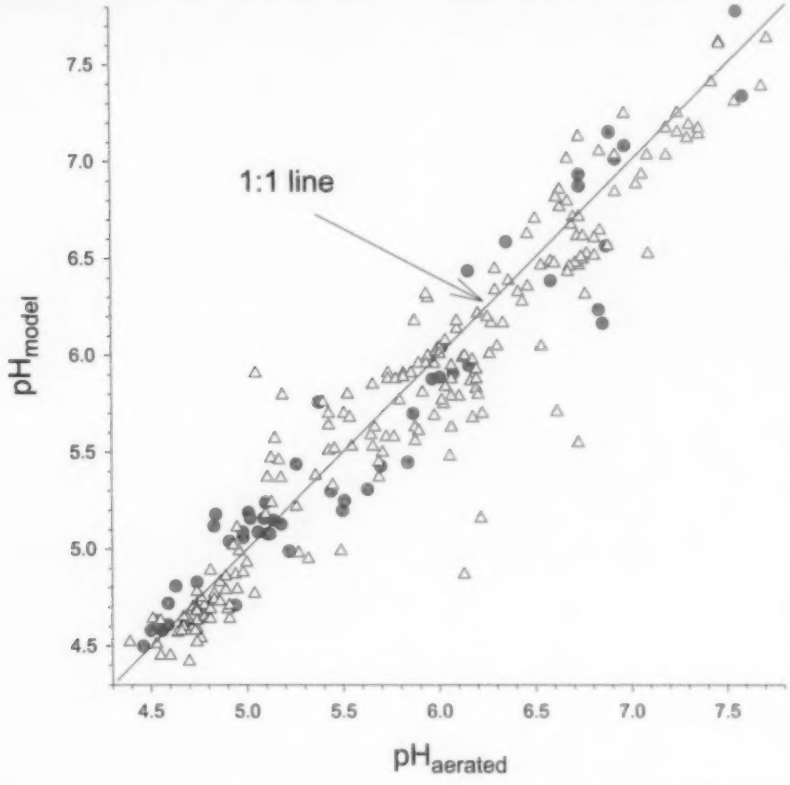
Fig. 5. pH prediction using the calibrated pH model in this study for the calibration and validation data sets.



TOC concentration had already begun to decrease when the peak discharge occurred, although not back to baseflow levels.

For all seven streams where total Al (Al_{total}) was measured, the concentration increased during the initiation of the increasing discharge. In all streams, total Al remained at a level well above the baseflow concentration during the melt season. There was a positive correlation between TOC and Al_{total} during the spring melt events (with an r^2 between 0.45 and 0.85 for the two melt seasons combined in the inland and coastal streams (Fig. 4)). In Fig. 4, a marked regional difference can be observed in the response to a changing TOC concentration between the coastal sites (Stridbäckan, Kälsbäcken, and Lillån) and the inland sites (Mellansjöbäcken and Sörbäcken). Whether this difference is due to climatic, hydrological, and geological variations or the difference in anthropogenic deposition remains to be explored. Four of the seven streams (the four most acid streams) experienced an increased concentration of labile monomeric Al associated with the increased flow and decline in pH. In all four streams, the labile monomeric Al increased at the onset of the spring runoff and remained at a stable level for the entire melt season until pH began to increase.

pH–pK_a relationship

The optimized pK_a (eq. 5) was calibrated against the effective pK_a (eq. 6). The 1997 spring runoff samples from the northern transect were used for that calibration, which represents approximately one fourth of the about 300 samples analyzed for the full suite of hydrochemical parameters in this study. A site density of 12 μequiv.·mg TOC^{-1} for the whole data set resulted from this calibration together with a set of optimized parameters, where $a = -1.95$, $b = 2.13$, and $c = -0.147$.

The calibration of the organic acidity using an Oliver et al. (1983) type model succeeded reasonably well in reproducing the measured air-equilibrated pH from ANC, pCO_2, and TOC using the 1997 stream water chemistry from the northern transect as a calibration data set and the remaining samples (about 210) for validating the model. The standard deviation for the calibration data set was 0.19 pH unit and for the validation set was 0.21 pH unit (Fig. 5). The error could be due to (*i*) analytical errors in TOC, cation, or anion determination, (*ii*) variation in the character of TOC, (*iii*) CO_2 disequilibria, or (*iv*) exclusion of other important protolytes.

Contribution of free acidity during spring melt runoff

Comparing the normalized contribution of acidity at minimum pH during the 1997 spring melt runoff for each of the study streams, using eqs. 8*a*–8*c*, the largest proton donor, in general, was organic acid, which provided over 75% of the acidity when pH reached its minimum (Tables 2 and 3). During the 1997 spring melt events, only the two Göljeån sites were exceptions to this overwhelming organic acid contribution. For Upper Göljeån, which is entirely above the tree

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Table 2. Normalized contribution of free acidity (protons) during spring melt runoff from organic acidity, SO_4^{2-}, and NO_3^-.

		Concentration (μequiv.·L^{-1})				Proton contribution (μequiv.·L^{-1})			
Stream	Dilution index	$RCOO^-$ (base–peak)	SO_4^{2-} (base–peak)	NO_3^- (base–peak)	$pH_{aerated}$ (base–peak)	$RCOO^-$	SO_4^{2-}	NO_3^-	ΔH^+ (μequiv.·L^{-1}) (peak–base)
1997 melt event									
Stridbäcken	0.43 (0.01)	57 (1) – 44 (2)	73 (12) – 37 (2)	BD–BD	5.46 (0.03) – 4.48 (0.08)	20 (2)	6 (1)	<1	29.4 (6.6)
Lillån	0.45 (0.01)	132 (10) – 100 (4)	132 (1) – 59 (6)	BD–BD	6.62 (0.03) – 4.93 (0.04)	40 (6)	<1	<1	11.5 (1.0)
Sörbäcken	0.47 (0.03)	90 (28) – 152 (19)	50 (1) – 32 (4)	BD–BD	7.52 (0.06) – 6.11 (0.17)	109 (39)	9 (1)	<1	0.7 (0.3)
Mellansjöbäcken	0.65 (0.02)	101 (3) – 111 (14)	31 (0) – 22 (0)	BD–BD	6.75 (0.05) – 5.11 (0.04)	45 (7)	2 (<1)	<1	7.6 (0.7)
Stopelebäcken	0.34 (0.05)	17–30 (3)	55–20 (4)	BD–BD	7.73–7.08 (0.14)	25 (5)	2 (<1)	<1	0.1 (<0.1)
Rukkebäcken	0.23 (0.02)	12–11 (2)	78–17 (1)	BD–BD	7.44–6.60 (0.09)	9 (2)	<1	<1	0.2 (<0.1)
Kälsbäcken	0.54 (0.06)	61–62 (2)	86–53 (5)	BD–BD	6.90–5.43 (0.03)	29 (4)	6 (1)	<1	3.6 (0.3)
Sörjabäcken	0.66 (0.12)	73 (1) – 2 (4)	83 (1) – 54 (14)	BD–BD	6.84 (0.03) – 5.99 (0.02)	4 (1)	<1	<1	0.9 (<0.1)
Acktjärnsbäcken	0.28 (0.02)	77 (3) – 70 (6)	27 (0) – 19 (2)	4 (1) – BD	7.34 (0.04) – 4.70 (0.04)	48 (7)	11 (1)	<1	19.9 (2.1)
Upper Göljeån	0.28 (0.12)	2 (1) – 14 (3)	36 (1) – 22 (5)	12 (1) – 4 (4)	6.71 (0.02) – 4.82 (0.14)	13 (7)	12 (5)	<1	14.9 (6.4)
Lower Göljeån	0.43 (0.03)	8 (2) – 17 (2)	39 (2) – 27 (2)	14 (1) – 4 (1)	7.06 (0.02) – 5.93 (0.10)	14 (5)	10 (1)	<1	1.1 (0.3)
1998 melt event									
Stridbäcken	0.65 (0.03)	69 (6) – 54 (8)	62 (1) – 51 (3)	BD–BD	5.47 (0.04) – 4.59 (0.04)	9 (2)	11 (1)	<1	22.3 (5.7)
Lillån	0.37 (0.04)	172 (1) – 102 (8)	95 (15) – 48 (4)	BD–BD	6.77 (0.06) – 4.53 (0.07)	39 (7)	13 (2)	<1	29.6 (4.5)
Sörbäcken	0.39 (0.03)	104 (15) – 159 (5)	41 (1) – 37 (0)	BD–BD	7.56 (0.05) – 6.06 (0.08)	118 (23)	21 (2)	<1	0.8 (0.2)
Mellansjöbäcken	0.53 (0.03)	135 (4) – 99 (4)	32 (0) – 24 (3)	BD–BD	6.93 (0.07) – 4.91 (0.07)	27 (4)	7 (1)	<1	12.2 (1.1)
Stopelebäcken	0.29 (0.01)	34–33 (6)	61–21 (1)	BD–BD	7.76–7.14 (0.01)	23 (5)	4 (<1)	<1	0.1 (<0.1)
Rukkebäcken	0.55 (0.02)	22 (0) – 19 (1)	38 (4) – 21 (3)	BD–BD	7.13 (0.03) – 6.87 (0.13)	7 (1)	<1	<1	0.1 (<0.1)
Kälsbäcken	0.45 (0.06)	59 (3) – 44 (1)	81 (4) – 62 (7)	BD–BD	6.87 (0.16) – 4.97 (0.03)	17 (3)	26 (4)	<1	10.7 (0.7)
Sörjabäcken	0.81 (0.08)	101 (7) – 141 (12)	80 (1) – 61 (3)	BD–BD	6.73 (0.17) – 5.96 (0.36)	59 (11)	<1	<1	0.9 (1.2)
Acktjärnsbäcken	0.34 (0.02)	128 (6) – 73 (3)	30 (2) – 20 (2)	1 (1) – BD	7.34 (0.04) – 4.70 (0.04)	29 (4)	10 (1)	<1	16.8 (1.4)
Upper Göljeån	0.90 (0.14)	4 (1) – 20 (1)	35 (1) – 44 (2)	4 (1) – 3 (1)	5.84 (0.06) – 4.57 (0.06)	16 (3)	12 (2)	<1	25.3 (3.5)
Lower Göljeån	0.53 (0.03)	26 (6) – 28 (1)	36 (3) – 24 (1)	2 (1) – 1 (1)	6.78 (0.03) – 6.15 (0.07)	15 (4)	5 (< 1)	<1	0.5 (0.1)

Note: The dilution index is the average dilution of BC from base flow to peak flow (eq. 7). Concentration and $pH_{aerated}$ denote the levels of average base flow and peak flow concentration for $RCOO^-$, SO_4^{2-}, NO_3^-, (BD, below detection limit) and aerated pH. Proton contribution is the maximum average contribution of H^+ provided from the individual acid anions to the decline in pH between base flow and peak flow calculated using eqs. 8*a* – 8*c*. ΔH^+ is the increase in free protons between base flow and peak flow. The parenthetical quantities of the dilution index, concentration, $pH_{aerated}$, and ΔH^+ are standard deviations. The parenthetical quantities of the proton contribution are the propagation of errors from all analyses, dilution, and the standard deviations in the average base flow and peak flow samples. All concentrations were calculated using three samples unless parentheses are missing, in which case the calculation consisted of only one sample. A lower pH (5.46) was measured at Sörjabäcken but was not analyzed for anions and cations. Since no baseflow sample was collected at Nyckelmyrbäcken prior to the onset of spring melt, the importance of the individual acids for the pH decline could not be estimated and is hence omitted from the table.

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Table 3. Relative contribution of protons (percentage) during spring melt runoff from $RCOO^-$, SO_4^{2-}, and NO_3^-.

	1997 spring melt			1998 spring melt		
Stream	$RCOO^-$	SO_4^{2-}	NO_3^-	$RCOO^-$	SO_4^{2-}	NO_3^-
Stridbäcken	76 (9)	24 (2)	0	46 (9)	54 (5)	0
Lillån	100 (14)	0 (2)	0	75 (13)	25 (3)	0
Sörbäcken	93 (33)	7 (2)	0	85 (17)	15 (1)	0
Mellansjöbäcken	96 (17)	4 (0)	0	80 (11)	20 (2)	0
Stopelebäcken	93 (20)	7 (1)	0	86 (18)	14 (1)	0
Rukkebäcken	100 (19)	0 (0)	0	100 (13)	0 (0)	0
Kälsbäcken	82 (13)	18 (2)	0	40 (7)	60 (9)	0
Sörjabäcken	100 (22)	0 (0)	0	100 (18)	0 (0)	0
Acktjärnsbäcken	81 (13)	19 (2)	0	75 (11)	25 (2)	0
Upper Göljeån	52 (30)	48 (2)	0	57 (11)	43 (7)	0
Lower Göljeån	59 (20)	41 (2)	0	75 (21)	25 (2)	0

Note: The parenthetical quantities of the proton contribution are the propagation of errors from all analyses, dilution, and the standard deviations in the average base flow and peak flow samples.

line, the SO_4^{2-} contribution was at the same level as the organic acidity. During the 1998 spring melt events, the contribution to the episodic pH decline was distributed more evenly between SO_4^{2-} and organic acids, but the latter still contributed most of the protons in the studied sites except for two of the coastal streams, Stridbäcken and Kälsbäcken (Tables 2 and 3). NO_3^- did not contribute to the pH decline during spring melt runoff, although detectable levels of NO_3^- during baseflow were observed in some streams.

Discussion

In general, TOC was the most important driving mechanism of the pH decline in most streams in this study. This is due to the large increase in TOC (and in $RCOO^-$) compared with the BC dilution (and dilution of most other constituents) during the spring melt events, despite significant inputs of snowmelt water into soils that were at least partly frozen. These results are in agreement with other spring melt event studies from boreal regions (Campbell et al. 1992; Laudon and Bishop 1999) showing that TOC often increases by 150–200% during snowmelt despite a dilution of most other solutes in the stream. Bishop and Pettersson (1996) suggested that much of the TOC dynamics in the boreal forest could be explained by release from new source areas that are "tapped into" during high-flow periods by the rise of the water table (and associated lateral flow pathways) into the organic-rich upper soil horizons. The subsequent decline in TOC as the spring melt progresses could therefore result from a depletion of these newly activated source areas.

A relative increase in the SO_4^{2-} ratio compared with that of BC presumably originates from anthropogenic acid deposition, but oxidation of bedrock and sediment sources could also contribute significantly to a deviation between the SO_4^{2-} and BC dilution during the episodes (Jansson and Ivarsson 1994). For most of the streams during the 1997 spring melt events in the northern transect, the SO_4^{2-} variation followed the dilution of BC during peak runoff reasonably well. This implies that the SO_4^{2-} level in the stream during most of the snowmelt period was not affected by acid deposition in the snowpack. Thus, the magnitude of the ANC and pH decline during 1997 was essentially that that would have been expected in preindustrial times. The largest exception to this general pattern is Sörbäcken, where the dilution of SO_4^{2-} was less than the decline in BC. Sörbäcken is, however, the catchment among the high-TOC streams in the northern transect with the highest peak flow pH (Fig. 6).

In the southern transect, the covariation between BC and SO_4^{2-} was less pronounced for most streams during the 1997 spring melt. For all streams, except Sörjabäcken, the dilution of BC was greater than the decline in SO_4^{2-}. The largest deviation between the decline in BC and SO_4^{2-} was found in the three catchments that are located above the highest postglacial marine level, where the baseflow SO_4^{2-} is the lowest of all the streams.

During the 1998 spring melt runoff in the northern transect, the dilution patterns for BC and SO_4^{2-} diverged more than was observed during the 1997 melt season. For most streams, the dilution of BC was larger than the dilution of SO_4^{2-}. This suggests that the higher deposition load during the winter of 1998 had a significant influence on the stream water chemistry. The influence of an increased anthropogenic SO_4^{2-} load in the 1998 runoff chemistry was probably enhanced by the rapid spring snowmelt that year. Another possible explanation of the larger divergence between BC and SO_4^{2-} during the 1998 spring melt could be that the fall of 1997 in the northern transect was relatively dry. A low groundwater level could have caused oxidation and subsequent mineralization of S already present in the catchment (Jansson and Ivarsson 1994). The between-year difference in the BC and SO_4^{2-} dilution was less pronounced in the southern transect except for Kälsbäcken where the 1998 dilution of SO_4^{2-} was much less than for BC.

NO_3^- concentrations were below the detection limit for all the study sites except Upper and Lower Göljeån in 1997 as well as in 1998 and during the 1997 baseflow at Acktjärnsbäcken. The baseflow concentrations of NO_3^- were considerably lower during the 1998 spring melt events compared with the previous year. At all sites with detectable NO_3^- in baseflow, the dilution of NO_3^- was larger than the BC dilution during the peak flow.

The negligible role of NO_3^- and the generally limited role of SO_4^{2-} in the spring pH declines monitored by this study contrast with several other studies of events with a similar

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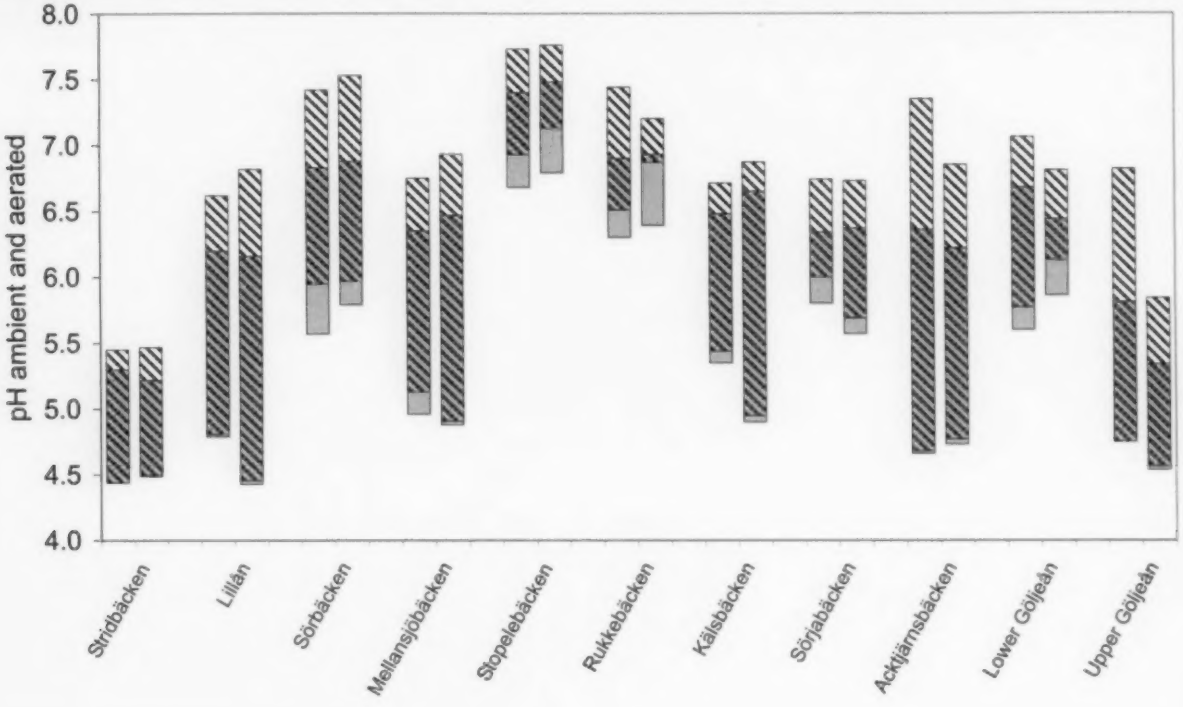
Fig. 6. Decline in aerated and ambient pH during the 1997 (first bar) and 1998 (second bar) spring melt events. The hatched portion of the bars denotes the variation in aerated pH. The gray portion of the bars denotes the ambient pH decline during the spring melt runoff.



magnitude in pH decline (Davies et al. 1992; Wigington et al. 1992; Wigington et al. 1996). In comparison with these previous studies, however, this study was conducted in a region with low deposition rates of SO_4^{2-} and NO_3^- in combination with generally low baseflow ANC levels and high concentrations of TOC that can naturally drive a pH decline during spring melt of over 2 pH units.

In studies by Laudon and Bishop (1999) and Laudon et al. (1999) during spring melt events in the same region, the most important driving mechanism of the ANC decline was found to be ANC dilution of the more well-buffered baseflow water with ionically weak snowmelt runoff. Although not directly quantified in this study, the dilution of BC (hence the dilution index) provides an estimate of the role of dilution in the natural ANC decline (Molot et al. 1989; DeWalle and Swistock 1994). The average BC dilution in the 22 spring melt events was 50%, making it an important contributor to the decline in the acid-buffering capacity. But since dilution per se does not produce any strong acidity, ANC can only be reduced towards zero. Thus, the negative effect of dilution on the pH is constrained to systems with pH values above 5.0–5.5 (depending on TOC concentration) with a positive ANC when the weak acids such as the carbonate system are diluted. In streams with a negative ANC, dilution will instead increase ANC, since the strong acid anions will be diluted in larger proportions than the buffering components (cf. eq. 1). Despite the low pH in many streams, only the Upper Göljeån site had a negative ANC during spring melt runoff.

Table 4. Average dilution of BC, Si, $RCOO^-$, and SO_4^{2-} during peak flow (average of the three consecutive samples that were most acid).

		Dilution			
Stream	Year	BC	Si	$RCOO^-$	SO_4^{2-}
Stridbäcken	1997	0.43	0.27	0.77	0.51
Stridbäcken	1998	0.65	0.38	0.78	0.82
Lillån	1997	0.45	0.43	0.76	0.45
Lillån	1998	0.37	0.28	0.59	0.51
Sörbäcken	1997	0.47	0.43	1.69	0.64
Sörbäcken	1998	0.39	0.38	1.53	0.90
Mellansjöbäcken	1997	0.65	0.39	1.10	0.71
Mellansjöbäcken	1998	0.53	0.40	0.73	0.75
Stopelebäcken	1997	0.34	0.34	1.76	0.36
Rukkebäcken	1997	0.23	0.25	0.92	0.22
Rukkebäcken	1998	0.55	0.60	0.86	0.55
Kälsbäcken	1997	0.54	0.51	1.02	0.62
Kälsbäcken	1998	0.45	0.46	0.75	0.77

Note: Only the streams where Si data were available are shown.

A potential problem using the BC concentration to define natural dilution during hydrological events is that BC could behave nonconservatively due to competition from other cations for exchange sites in the soil. Since the relative concentration of anions, compared with the BC concentration, increases during the spring melt, it is possible that some BC (instead of protons as the model assumes) will accompany

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anions to the stream and hence not dilute conservatively. Of specific concern for this study is the possibility that increased TOC (and hence, organic anions) during spring melt events would cause a natural increase in BC (Kahl et al. 1992; Köhler 1999), relative to what would be expected from dilution of baseflow.

One way of examining whether or not organic anions (as well as mineral anions) affect the BC concentration in runoff is to compare the relative dilution of BC and Si. Silica has been shown to behave conservatively during hydrological events, under certain conditions, and hence dilute in proportion to the amount of snowmelt water input routed as overland flow or through the upper soil organic horizons (Hooper and Shoemaker 1986; Wels et al. 1991). Of the 13 episodes where Si data were available, five episodes (1997 and 1998 spring melt events from Stridbäcken and Mellansjöbäcken together with the 1997 spring melt from Lillån) displayed a discrepancy between the BC and Si dilution at peak flow larger than 5% (Table 4). This might be interpreted as a sign that these streams have BC transported by TOC, thus giving less BC dilution relative to Si when TOC (and $RCOO^-$) increases with increasing flow. These five episodes, however, have the smallest relative increases in $RCOO^-$ of all the streams (Table 4). This appears to minimize the importance of TOC for BC transport during spring melt. Instead, the discrepancy in the dilution patterns of Si and BC appears more likely to be related to wetland area, since the three catchments with the largest BC and Si dilution discrepancy also have the highest percentage of wetland area of all of the study catchments. There is probably a larger source limitation of Si in wetland areas (since Si is a mineral weathering product) than for BC (derived mainly from cation-exchange reactions in mineral and organic soils). Thus, large mire complexes could cause the discrepancy in the Si and BC dilution patterns found in this study. Furthermore, studies in Canada have observed that wetland areas act as a source of Si during baseflow and as a sink during spring melt (Wels et al. 1991), which calls into question the use of Si as a dilution index in such catchments.

Another potential problem in using the BC dilution to define the natural dilution of acid anions is that the different BC (Ca^{2+}, Mg^{2+}, Na^+ and K^+) do not dilute uniformly due to variation in sources. Munson and Gherini (1993) suggested that the source of the divalent ions is mainly cation exchange in the soil, whereas the principal source of Na^+ is mineral weathering and sea salt deposition in coastal areas. The main source of K^+ is degradation of organic material. The divalent cations in all 22 spring melt episodes are the main component of BC and are thus the most important factor in BC dilution. In most events, K^+ dilutes less than the sum of BC. Since K^+ usually constitutes less than 10% of BC, that ion does not substantially affect the BC dilution. In six of the 22 events, Na^+ dilution differed more than 5% from the dilution pattern of BC. In 1998 at the two Göljeån sites, Na^+ diluted to a larger extent than BC, while in Stopelebäcken, Rukkebäcken, and Kälsbäcken in 1997 and in Rukkebäcken in 1998, Na^+ diluted less than BC. However, in the latter events, the BC dilution correlates well with the dilution of Si (Table 4).

In conclusion, the results from this study suggest that natural organic acids and dilution of the buffering capacity play an important role in depressing the spring melt buffering capacity and pH in the region. In only three of the more than 22 events studied was the anthropogenic contribution to pH decline as large as the natural contribution. The interannual variation between the 1997 and 1998 spring melt events was probably driven by the higher anthropogenic deposition load during the winter of 1998 enhanced by the rapid runoff during the spring melt.

The study also shows that the sampling methodology used provides useful information for understanding the role of anthropogenic versus natural acidity during pH decline associated with the spring melt. Routine monitoring of the spring melt runoff chemistry would provide the means to better establish the relationship between winter acid deposition and spring melt pH decline. While further quantification of the human impact on the spring melt runoff chemistry is important, it is clear that a substantial pH decline during spring melt episodes is a natural feature of the boreal ecosystem and not only a result of acid deposition.

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Spring isothermal mixing in the Great Lakes: evidence of nutrient limitation and nutrient–light interactions in a suboptimal light environment

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Abstract: During the spring isothermal mixing period (April–May) in 1993–1995, photosynthesis–irradiance and growth–irradiance experiments were conducted in Lakes Erie, Huron, Michigan, and Ontario to assess light limitation. Additionally, nutrient enrichment experiments were conducted in Lake Ontario. Results from the photosynthesis–irradiance experiments suggested that phytoplankton communities in all the lakes can be either light limited or light saturated, as the threshold parameter (I_k) was similar to mean water column irradiances ($\bar{I}_{wc}$, ratio = 1.0). Growth–irradiance experiments also suggested the potential for light saturation; mean daily irradiance exceeded the threshold growth irradiance ($I_{k,g}$) in 95% of cases. Growth rates became light saturated at lower irradiances than photosynthetic rates. Evidence for a nutrient–light interaction in controlling in situ growth rates was also found in the nutrient enrichment experiments at incubation irradiances $\geq \bar{I}_{wc}$. Our results suggest that an interaction between nutrients and light is often controlling phytoplankton growth during spring mixing in the Great Lakes. The role of these nutrient–light interactions has increased in the past decade due to increased light availability in the lower lakes caused by phosphorus load reductions and the filtering activities of nonindigenous mussels.

Résumé : Pendant la période printanière de brassage isotherme (avril–mai), en 1993–1995, nous avons réalisé dans les lacs Érié, Huron, Michigan et Ontario des expériences sur les rapports photosynthèse – éclairement énergétique et croissance – éclairement énergétique pour évaluer la limitation par l'éclairement. De plus, nous avons mené des expériences d'enrichissement en nutriants dans le lac Ontario. Les résultats des expériences photosynthèse – éclairement énergétique permettent de penser que les communautés phytoplanctoniques de tous les lacs peuvent subir soit une limitation soit une saturation par la lumière, car le paramètre seuil (I_k) était semblable à l'éclairement énergétique moyen de la colonne d'eau ($\bar{I}_{wc}$, rapport = 1,0). Les expériences croissance – éclairement énergétique semblaient aussi indiquer un potentiel de saturation par la lumière; l'éclairement énergétique moyen dépassait le seuil de croissance ($I_{k,g}$) dans 95% des cas. La saturation par la lumière était atteinte pour les taux de croissance à des niveaux d'éclairement énergétique plus bas que pour la photosynthèse. Nous avons aussi constaté dans les expériences sur l'enrichissement que l'interaction nutriants–lumière régit les taux de croissance in situ à des niveaux d'éclairement énergétique $\geq \bar{I}_{wc}$ pendant l'incubation. Nos résultats permettent de penser qu'une interaction entre les nutriants et la lumière régit souvent la croissance du phytoplancton pendant le brassage printanier dans les Grands Lacs. Le rôle de ces interactions nutriants–lumière a augmenté au cours de la dernière décennie à cause de la hausse de l'éclairement dans les lacs d'aval liée à la baisse des charges en phosphore et aux activités de filtrage des moules exotiques.

[Traduit par la Rédaction]

Introduction

In many environments, the availability of light controls phytoplankton photosynthesis and growth. The environments where light availability may limit growth are highly diverse, ranging from turbid estuaries (Pennock 1985; Alpine and Cloern 1988) to deep clear-water lakes (Brooks and Torke 1977; Nalewajko and Voltolina 1986). The St. Lawrence Great Lakes are one such environment. Because of the depth of these lakes, during periods of complete water column mixing, phytoplankton communities spend most of their time in the aphotic zone. In the spring isothermal mixing period, the ratio of the euphotic zone to water column depth typically is less than 0.5 in all the lakes (Table 1). During this period, light has been suggested as limiting phytoplankton growth in Lakes Superior (Nalewajko and Voltolina

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Table 1. Station depth (SD), euphotic zone depth (EZD), EZD:SD ratio, mean irradiance in the mixed layer expressed as a percentage of surface incident irradiation (% $\bar{I}_{Z_m}$), and summary photosynthetic characteristics (P_{max} (mg C·mg Chl^{-1}·h^{-1}), α (mg C·mg Chl^{-1}·mol quanta^{-1}·m^{-2}), and I_k (mol quanta·m^{-2}·h^{-1})) (all mean values) at sampling stations.

Station	SD (m)	EZD (m)	EZD:SD	% $\bar{I}_{Z_m}$	P_{max}	α	I_k
Lake Michigan south	107	21	0.20	4.3	1.32	10.0	0.16
Lake Michigan north	144	24	0.17	3.6	1.70	11.9	0.16
Lake Huron north	64	26	0.41	8.8	0.95	7.2	0.14
Lake Huron central	68	27	0.40	8.6	1.26	8.6	0.15
Lake Huron south	37	25	0.68	14.7	1.39	8.8	0.16
Lake Erie east	57	23	0.40	8.8	2.51	12.0	0.22
Lake Ontario west	133	25	0.19	4.1	1.60	12.2	0.14
Lake Ontario east	159	26	0.16	3.6	1.51	9.0	0.19

1986), Ontario (Lean et al. 1987; Millard et al. 1996), Erie (Lean et al. 1983), and Michigan (Brooks and Torke 1977). Light limitation may even occur in the surface mixed layer during thermal stratification (Nalewajko et al. 1981). Because of the primary importance of light to phytoplankton growth, the value of phosphorus control in maintaining and controlling phytoplankton biomass has been questioned (Nalewajko et al. 1981).

During the past decade, significant changes have occurred in the water quality of the Great Lakes due to phosphorus load reductions and the filtering activities of nonindigenous mussels (Johengen et al. 1994; Fahnenstiel et al. 1998). Large increases in transparency have been noted in the lower Great Lakes (Fahnenstiel et al. 1998). This increased light availability would not only affect phytoplankton growth rates during periods of light limitation, but may even produce nutrient limitation, or a nutrient–light interaction (Rhee and Gotham 1981; Fahnenstiel et al. 1984) where light limitation existed in the past. Thus, simple light limitation during spring isothermal mixing may not be as common in the 1990s, particularly the lower Great Lakes, as it was in the 1980s. The intent of this research was to examine the roles of nutrients and light during spring isothermal mixing in the Great Lakes in the early to mid-1990s, with particular emphasis on the potential for nutrient limitation.

In this study, as in all studies of nutrient and light limitation, the distinction between rate and biomass limitation must be emphasized. In laboratory chemostats of algae, the distinction between growth rate and biomass limitation are clear, but for field communities of phytoplankton, it is more difficult. Rate limitation is easier to document for field communities than biomass limitation. Nutrients can limit biomass and rates. Light is primarily a rate-limiting factor but can limit biomass when light levels are very low and self-shading becomes an important concern (i.e., turbid lake). Temperature is also primarily a rate-limiting factor. In natural communities, light and temperature can indirectly limit biomass through their control over growth rates, as the balance between growth and loss rates is an important factor controlling in situ phytoplankton biomass (Wofsy 1983). In our experiments, we focused on examining the role of light in limiting rates of photosynthesis and growth. If phytoplankton were found to be light saturated at in situ light levels, then other factors (nutrients, temperature, etc.) may potentially be limiting. Additionally, nutrient enrichment experiments were conducted to examine the potential for nutrients to control both rates and biomass, as the large increase in nutrient concentration added as part of the enrichment can increase both rates and biomass. While biomass limitation can be elucidated with nutrient enrichment experiments, the large nutrient perturbation sometimes reduces the applicability to in situ conditions.

Methods

Sampling was conducted at eight stations in the St. Lawrence Great Lakes aboard the RV *Laurentian* (Fig. 1). Two or three stations in the offshore region of each lake were sampled during the spring isothermal period (April–May) of each year from 1993 to 1995. Each station was occupied for 3–24 h.

At each station, a Seabird CTD (conductivity, temperature, and depth meter equipped with Sea-Tech fluorometer and transmissometer, 25-cm beam path) cast was made from the surface to just above the bottom at a 2-Hz sampling rate. The CTD profiles were used to determine the discrete sampling depths for water collections. Secchi disk transparency was measured with a black/white 25-cm disk. Underwater light extinction of photosynthetically active irradiation (k_{PAR}) was measured with a LICOR 193SB scalar (4π) light sensor and LICOR 1000 data logger and (or) a Biospherical integrating natural fluorometer (INF-3000) equipped with a downwelling scalar PAR sensor located on the ship. Surface incident irradiation was measured on the ship using a LICOR sensor and data logger. At stations sampled in darkness, beam attenuation values were converted to extinction coefficients using the conversion of Fahnenstiel et al. (1995), developed in Saginaw Bay with the same transmissometer. These k_{PAR} values were used to calculate the depth of the euphotic zone (1% isolume).

Discrete samples were taken from three to six depths in the euphotic zone using a modified Niskin bottle (all rubber parts were replaced with silicone- or teflon-coated parts) and poured into a 24-L carboy (one carboy for each depth). All water samples were taken from these carboys. Chlorophyll samples were filtered onto Whatman GF/F filters, extracted with *N,N*-dimethylformamide, and analyzed fluorometrically (Speziale et al. 1984).

Phytoplankton samples were preserved in amber bottles with 0.5% Lugol's solution. These samples were filtered onto microscope slides according to the procedure of Dozier and Richerson (1975). A minimum of 300 phytoplankton entities were enumerated under both high (1000–1200×) and low magnification (200×). Cell volumes were estimated by determining average cell dimensions of a minimum of 100 cells for each dominant taxon and at least 10 cells for rare taxa and then applying these dimensions to appropriate geometric shapes. The cell volumes used for this study were from a compilation of values from this and previous studies

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Fig. 1. Location of sampling stations.

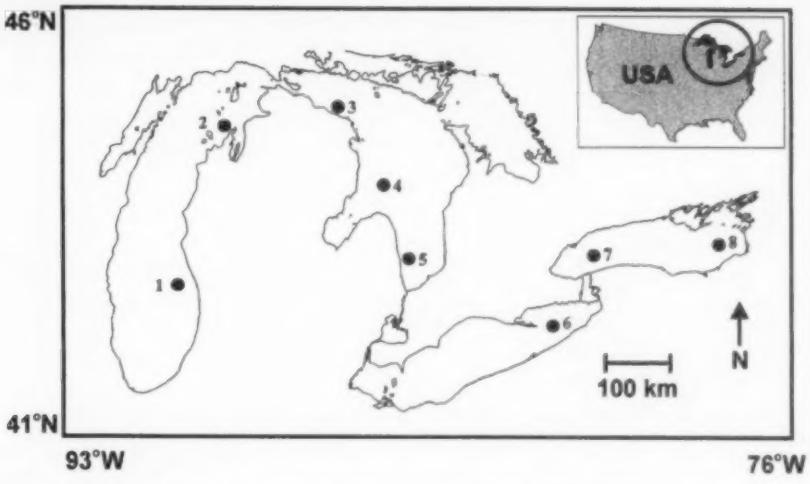
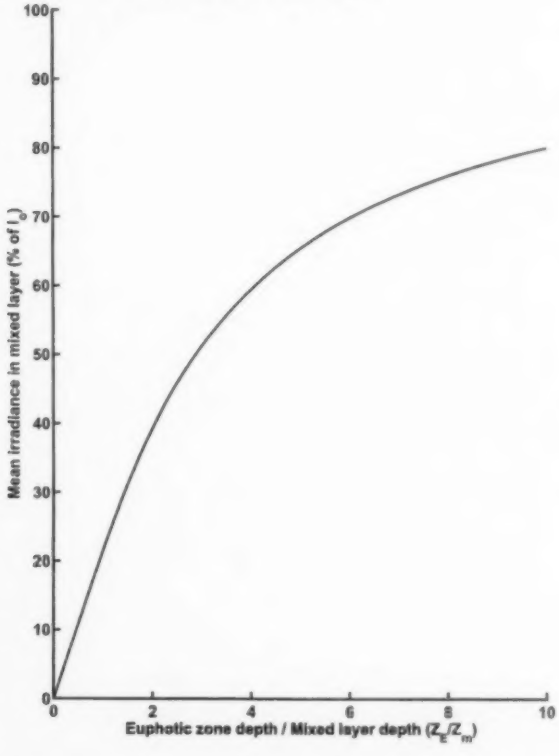


Fig. 2. Relationship between mean irradiance in the mixed layer expressed as percentage of incident irradiation (I_0) and the ratio of euphotic zone depth to mixed layer depth.



of the Great Lakes. Phytoplankton volumes were converted to carbon units using the respective equations from Strathman (1967) for diatoms and from Verity et al. (1992) for nondiatoms.

Phytoplankton photosynthesis was measured with the ^{14}C technique in a photosynthetron (Fahnenstiel et al. 1995). Water samples were inoculated with ^{14}C, and subsamples of 3 mL were incubated in acid-rinsed scintillation vials for 40 min. Eighteen light levels from <1 to 1800 mol quanta·m^{-2}·s^{-1} were used. After incubation, subsamples were acidified and sparged with air for 15 min. Time-zero blanks were taken and subtracted from all light values. Total carbon dioxide was determined from alkalinity and pH measurements.

Photosynthetic rates, normalized to chlorophyll, were used to construct a single photosynthesis–irradiance curve using the methods outlined in Fahnenstiel et al. (1989). Three parameters were determined from this model: P_{max}, maximum photosynthetic rate at light saturation (milligrams carbon per milligram chlorophyll per hour); α, initial linear slope at low irradiances (milligrams carbon per milligram chlorophyll per mole quanta per square metre); β, negative slope at high irradiance (same units as α). If the 95% confidence interval of β included zero, a simple two-parameter model was used (Fahnenstiel et al. 1989). The light saturation parameter (I_k), which has been used to differentiate between the light-limited and light-saturated regions of photosynthesis (Millard et al. 1996), was determined as the ratio P_{max}:α.

Phytoplankton growth rates were determined by ^{14}C labeling into chlorophyll *a* (Redalje and Laws 1981; Goericke and Welschmeyer 1993). This technique is basically an extension of the traditional ^{14}C experiment except that the end-point is labeling into chlorophyll *a*. The chlorophyll-specific growth rate equals the carbon-specific growth rate when growth is balanced. Significant photoadaption may decouple carbon and chlorophyll synthesis. To minimize photoadaptation, all experiments were conducted for 24 h (dawn to dawn) and incubated at as close to in situ irradiances as possible with static bottle incubations. These type of precautions should minimize photoadaptation artifacts and provide reasonable estimates of growth based on chlorophyll synthesis (Goericke and Welschmeyer 1993; Gallegos and Vant 1996). For our growth rate experiments, water samples were collected 1–4 h before dawn. Clean 2-L samples were incubated with 400 µCi of ^{14}C (1 µCi = 37 GBq) and placed in an in situ simulated incubator where the spectral quality of light was similar to in situ conditions. Six incubators with different light levels (50, 25, 12, 6, 3, and 1% of surface irradiance) were used to simulate the quantity of light received at depth. All growth samples were incubated at the irradiance that most closely approximated in situ irradiance. In most cases (57%), the samples were collected from the depth that received mean water column irradiance (Fig. 2) and then incubated at this mean water column irradiance. These incubations will be referred to as simulated in situ incubations. The other cases (43%) are where samples were collected at specific depths and then incubated at the irradiances received at those specific depths. Chlorophyll *a* was ex-

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tracted and analyzed for radiochemical activity using the techniques of Redalje (1990). Dark and time-zero controls were performed. Growth rates were calculated from the specific activity of chlorophyll *a* using eq. 6 from Goericke and Welschmeyer (1993). By combining growth rates from all experiments over a 3-year period as incident irradiation varied, a growth–irradiance curve was constructed for each lake. These growth–irradiance curves were then fit with a nonlinear model similar to that used for photosynthesis–irradiance curves described above. Two parameters were determined from these models: μ_{max}, maximum growth rate at light saturation; α_g, initial linear slope at low irradiances. From these two parameters, a light saturation parameter ($I_{k,g}$) was calculated as the ratio μ_{max}:α_g.

On eight occasions, phytoplankton growth rates were also estimated with the dilution technique (Landry and Hassett 1982). These experiments consist of a series of incubations in which raw lake water (unfiltered) is diluted with varying amounts of filtered water; the phytoplankton growth rate is the intercept of the growth rate from each dilution regressed against the fraction of raw lake water. Diluted samples (2- to 20-fold) were prepared by mixing appropriate volumes of raw lake water with filter sterilized lake water (<0.2 μm) and incubating in 4-L polycarbonate bottles. Because only growth rates were determined, more highly diluted samples (8- to 20-fold dilutions) were used. Water for these dilution experiments was taken from the depth at which the mean irradiance in the water column was received (Fig. 2). All dilution samples were incubated in the simulated in situ incubator described above at the mean irradiance in the mixed layer and at lake water temperature. Samples for phytoplankton counts were removed at 0, 48, and 72 h. Growth rates were calculated from changes in phytoplankton carbon determined from microscopic counts.

Nutrient enrichment experiments were conducted with water samples from Lake Ontario (Station 7 or 8). For these experiments, water was incubated in 20-L carboys in a simulated in situ incubator to assess the potential for nutrient stimulation of phytoplankton growth and biomass. These samples were maintained at ambient temperature and light level (2–12% of incident irradiation). Incubator temperature was maintained within 1°C of lake water temperature. Two experiments were conducted, and for each experiment, two light levels were used. For the spring 1993 experiment, light levels were 2 and 6% of incident, whereas for the spring 1994 experiment, light levels were 4 and 12% of incident. This range of light intensities allowed us to examine the potential for light limitation in a range of naturally occurring irradiances during the spring isothermal period. A complete set of macronutrients (phosphorus, nitrogen, and silicon) and micronutrients (vitamins and trace metals) were added in order to alleviate nutrient limitation. The concentrations of added macronutrients were 0.5 μM phosphorus, 8 μM nitrogen, and 25 μM silicon. The vitamins and trace metals were supplied in proportion to phosphorus based on WC media (Guillard and Lorenzen 1972).

Calculations for light parameters were as follows. Irradiance in the water column at a specific depth (I_Z) can be determined by

$$(1) \qquad I_z = I_0 e^{-kZ}$$

where k is the extinction coefficient of PAR, I_0 is surface irradiance, and Z is depth. Mean irradiance in the mixed layer ($\bar{I}_{Z_m}$) can be calculated by integrating eq. 1 over the mixed layer:

$$(2) \qquad \bar{I}_{Z_m} = \frac{I_0}{kZ_m}[1 - e^{-kZ_m}]$$

where Z_m is the depth of the mixed layer. For the spring isothermal mixing period in the Great Lakes, the mixing depth equals the station depth or water column depth ($\bar{I}_{Z_m} = \bar{I}_{we}$). Assuming that the euphotic zone depth (Z_E) equals the depth of 1% of I_0, then eq. 2 can be solved for the mean irradiance in the mixed layer as a percentage of surface incident irradiance ($\%\bar{I}_{Z_m}$):

$$(3) \qquad \%\bar{I}_{Z_m} = 21.7156\left(\frac{Z_E}{Z_m}\right)\left[1 - e^{-4.605\left(\frac{Z_m}{Z_E}\right)}\right].$$

Using this equation or Fig. 2, the mean irradiance in the mixed layer as a percentage of surface irradiance can be related to the ratio of the euphotic zone depth to mixed layer depth. Even when the euphotic zone equals the mixed layer, phytoplankton receive approximately 22% of surface irradiance.

Results

The sampling periods during each year corresponded to spring isothermal mixing (late April – May). For spring 1993 and 1994, all stations exhibited isothermal conditions with temperatures between 1 and 4°C. Sampling occurred 2 weeks later in May of 1995 than in 1993 and 1994, and slight stratification was found at two stations that were isothermal in previous years. Surface temperatures at the southern Lake Huron station and eastern basin of Lake Erie were 5°C, whereas those at all other stations in Lakes Huron, Michigan, and Ontario ranged between 2.5 and 4°C. The results from these two stations were not included in any analysis.

The euphotic zone was relatively similar among all stations sampled, ranging from 21 to 27 m (Table 1). The ratios of euphotic zone depth (EZD) to station depth (SD) were more variable, ranging from 0.16 to 0.68. These differences were strongly related to the station depth, as deeper stations (Lake Ontario and northen Lake Michigan) had the lowest ratios. Using eq. 3 or Fig. 2 and the EZD:SD ratios, the percentage of surface irradiance received in the water column during spring isothermal mixing ranged from 4 to 15% (Table 1).

Isothermal conditions do not necessarily imply vertically homogeneous distributions of phytoplankton, and this condition is important for our analysis. For all of our isothermal samplings, phytoplankton carbon and chlorophyll concentrations were uniform with depth ($p > 0.05$). In all cases, chlorophyll concentrations varied by <10% throughout the water column and phytoplankton carbon by <20%. Moreover, the physiological condition of phytoplankton communities in the isothermal water column was also similar with depth. Photosynthesis–irradiance parameters were measured with depth at several stations and no significant differences were noted ($p < 0.05$). In vivo fluorescence profiles from the CTD casts can also be used to examine the homogenous distributions of phytoplankton, and possibly even their physiological conditions. Because fluorescence yields can vary over short time periods (minutes), the fluorescence profile represents phytoplankton abundance and photoacclimation. In most cases, in vivo chlorophyll fluorescence was uniform with depth (82% of profiles). Even for profiles where statistical differences were noted (18%), chlorophyll fluorescence varied by <10% in the water column. Thus, during all of our samplings, phytoplankton biomass and physiological condition were vertically uniform.

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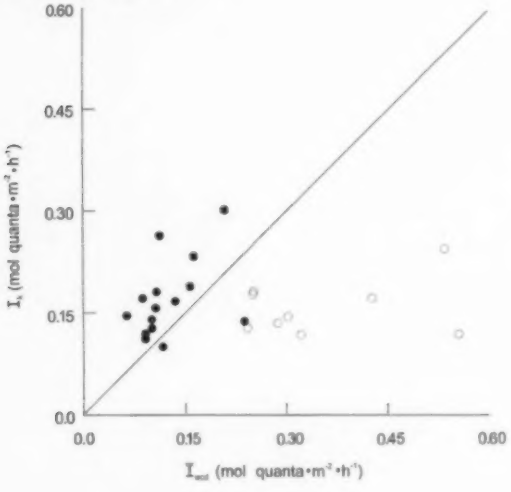
Fig. 3. Relationship between the light saturation parameter for photosynthesis (I_k) and mean daylight water column irradiance ($\bar{I}_{wcd}$) calculated for the preceeding 3 days. Open circles indicate Lake Huron data.

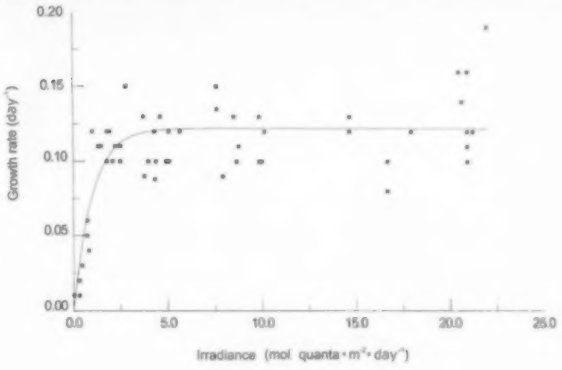


Photosynthetic rate parameters were relatively similar among stations (Table 1). The P_{max} values ranged from 0.95 to 2.51 mg C·mg Chl^{-1}·h^{-1}, with significantly lower values associated with Lake Huron stations ($p < 0.05$). The α values were similar across all stations, ranging from 7.2 to 12.2 mg C·mg Chl^{-1}·mol quanta^{-1}·m^{-2} ($p > 0.05$). The threshold parameter I_k was also similar among all stations, ranging from 0.14 to 0.22 mol quanta·m^{-2}·h^{-1} ($p > 0.05$).

In our study, comparisons of I_k and mean in situ irradiance ($\bar{I}_{wcd}$, calculated as mean water column irradiance during the daylight period) were used to determine light-limited conditions for spring phytoplankton (Fig. 3). A 3-day mean $\bar{I}_{wcd}$ was used to average out any single-day variation and to be more consistent with the generation times of the phytoplankton. Photosynthesis for natural phytoplankton communities was found to be both light limited and light saturated in the spring, as the ratio I_k:$\bar{I}_{wcd}$ was both above and below 1 but strongly dependent on the EZD:SD ratios. Those communities with relatively high EZD:SD ratios (all Lake Huron values and one Lake Erie value) exhibited $\bar{I}_{wcd}$ values greater than I_k (right-hand side of plot). On the other hand, phytoplankton communities with lower EZD:SD ratios (all Lakes Michigan and Ontario values and two Lake Erie values) exhibited I_k values that were higher than $\bar{I}_{wcd}$ values, thus suggesting light limitation. Because many values from all lakes were close to 1, the potential for both light limitation and saturation cannot be dismissed. For example, even though all Lake Michigan ratios were >1, the mean of all ratios was only 1.3.

Growth rates determined from the synthesis of chlorophyll *a* were relatively similar among the lakes, ranging from 0.01 to 0.19·day^{-1} (Figs. 4 and 5). Maximum growth rates (μ_{max}) were similar for each lake, ranging from 0.12 to 0.16·day^{-1} ($p > 0.05$). Even though the initial linear slopes ($I_{k,g}$) were also similar among lakes ($p > 0.05$, range 0.08–0.15 m^2·mol quanta^{-1}), limited sampling in the low-light region prevented any thorough comparison.

Fig. 4. Growth rate versus incubation irradiance for all experiments.



Precautions were taken to ensure that our chlorophyll synthesis growth rates were similar to carbon-based growth rates: 24-h incubations started at dawn, incubation at in situ irradiances, etc. Because the generation time of Great Lakes phytoplankton was long (mean = 6.3 days) relative to the incubation duration (24 h), it is likely that phytoplankton were adapted to a mean water column irradiance and that significant photoadaption did not occur during our relatively short incubations. To evaluate the effectiveness of these precautions and whether our growth rates were similar to carbon-based growth rates, on eight occasions, we compared chlorophyll synthesis growth rates with dilution growth rates calculated from changes in phytoplankton carbon. No significant differences were found between chlorophyll and dilution growth rates ($p > 0.05$; mean dilution = 0.14·day^{-1}, mean chlorophyll = 0.12·day^{-1}), suggesting that the assumption of balanced growth was met and that our chlorophyll synthesis growth rates are reasonable measures of the growth rate. These dilution growth rates were used only for comparative purposes and are not included in any further analysis.

The potential for light limitation was also examined in relation to phytoplankton growth rates (Figs. 4 and 5). The light saturation parameter for all growth rates, $I_{k,g}$, was 1 mol quanta·m^{-2}·day^{-1} (μ_{max} = 0.12·day^{-1}, α_g = 0.12 m^2·mol quanta^{-1}; R^2 = 0.66, $p < 0.05$) (Fig. 4). For the individual lakes, $I_{k,g}$ values were higher for Lakes Erie and Huron (1.6 and 1.5 mol quanta·m^{-2}·day^{-1}, respectively) than for Lakes Michigan and Ontario (1.0 and 0.9 mol quanta·m^{-2}·day^{-1}), respectively. Growth rates became light saturated at irradiance of around 3–4 mol quanta·m^{-2}·day^{-1}. Mean water column irradiance ($\bar{I}_{wc}$, calculated for a 24-h period) exceeded $I_{k,g}$ values in almost all cases (95% of days) and the light saturation level (4 mol quanta·m^{-2}·day^{-1}) on 26% of days. These data suggest light saturation of growth was common at in situ irradiances. Growth versus simulated irradiance curves for the individual lakes also demonstrate the potential for light saturation (Fig. 5). The majority of simulated in situ growth rates were near μ_{max}, and simulated irradiances were greater than $I_{k,g}$ values. However, because many of the growth rates were close to $I_{k,g}$, the possibility of either light limitation or light saturation exists (Fig. 5).

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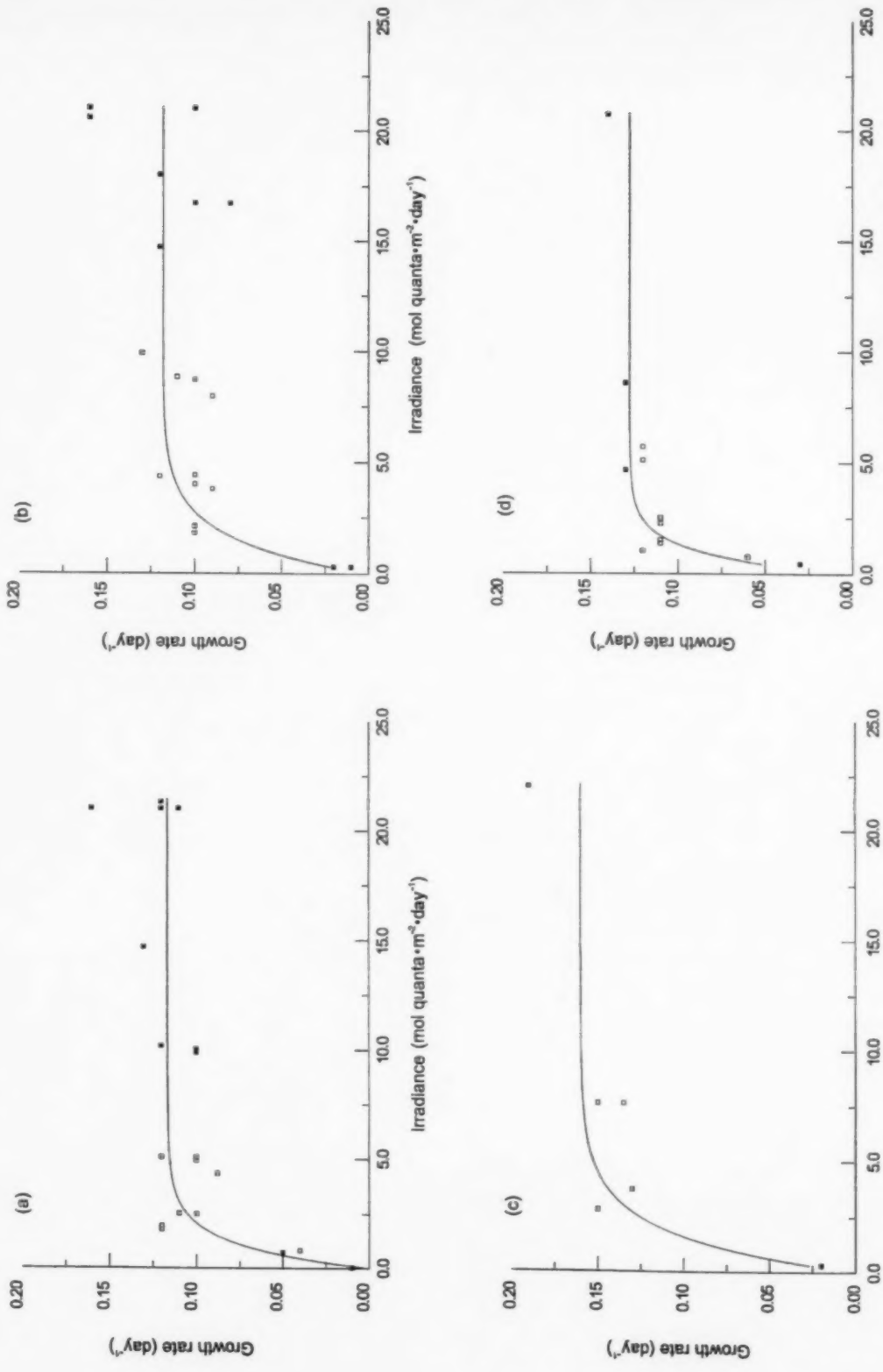

Fig. 5. Growth rate versus incubation irradiance for (*a*) Lake Michigan, (*b*) Lake Huron, (*c*) Lake Erie, and (*d*) Lake Ontario. Open squares are for growth experiments incubated at mean water column irradiance.

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Fig. 6. Chlorophyll concentrations from May 1993 nutrient enrichment experiment. Experiments were performed at 2% (circles) and 6% (squares) of surface irradiance. Solid symbols indicate nutrient treatments.

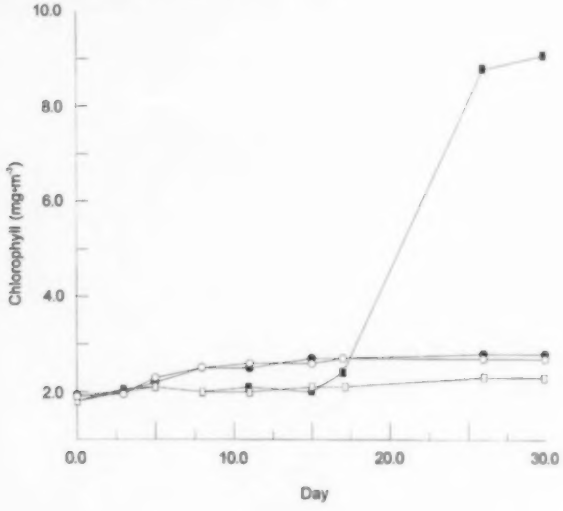
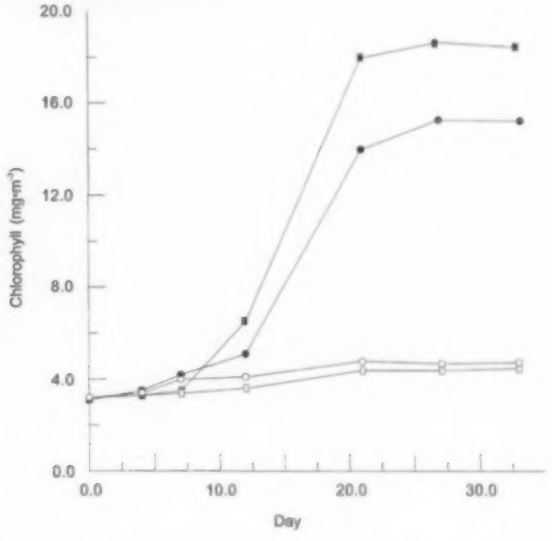


Fig. 7. Chlorophyll concentrations from May 1994 nutrient enrichment experiments. Experiments were performed at 4% (circles) and 12% (squares) of surface irradiance. Solid symbols indicate nutrient treatments.



Lakes Ontario and Michigan had a greater percentage of simulated in situ growth rates in the light-limited region of the curve than did Lake Erie or Lake Huron.

Nutrient enrichment experiments demonstrated the potential for nutrient limitation in Lake Ontario. In the May 1993 experiment, at the 2% of surface irradiance treatment, both control and nutrient treatments exhibited similar biomass throughout the experiment (Fig. 6). For the 6% light treatment, a significant increase in biomass was noted at day 26 ($p < 0.05$), as chlorophyll concentration increased almost four times higher than controls. Even though biomass increased only slightly by day 17, growth rates and P_{max} values for the nutrient treatment were significantly greater (about 50%, $p < 0.05$) than control values on day 17. In the May 1994 experiment, a significant nutrient effect was noted for nutrient treatments at both the 4 and 12% light levels ($p < 0.05$) (Fig. 7). On day 7, significant increases in growth rates for nutrient treatments were noted at the 12% (40% increase, $p < 0.05$) and 4% (30% increase, $p < 0.05$) light levels. The results from these experiments suggest that the threshold for nutrient limitation was >2 but <4% of surface irradiation. The actual incident surface irradiation during these two experiments was 38 and 39 mol quanta·m^{-2}·day^{-1}, respectively. Thus, the absolute threshold for these two experiments was between 0.8 and 1.6 mol quanta·m^{-2}·day^{-1}, which is very similar to the light saturation parameter value for growth of 1 mol quanta·m^{-2}·day^{-1}.

The consistency of phytoplankton biomass in the control treatments is noteworthy and suggests that the results may be applicable to in situ conditions. In the May 1993 experiment, chlorophyll concentrations in the controls changed only 25 and 40% after 30 days (Fig. 6). Moreover, phytoplankton growth rates in the controls were not significantly different after 27 days ($p < 0.05$). In the May 1994 experiment, chlorophyll concentrations changed only 30–40% after 33 days (Fig. 7). Also, photosynthetic and growth parameters were similar in controls after 21 days ($p < 0.05$).

Discussion

Phytoplankton photosynthesis and growth during spring isothermal mixing in the Great Lakes are not strictly controlled by the availability of light. In many cases, phytoplankton growth and, to a lesser extent, photosynthesis rates were at light saturation, suggesting that factors other than light (nutrients, temperature, etc.) may be important in controlling rates. Although we did not sample Lake Superior, the similarity of EZD:SD ratios between Lake Superior and the other lakes suggests that other factors may also be important in Lake Superior. Lake Superior has a greater euphotic zone (about 42–46 m; Fahnenstiel et al. 1998) and greater depths (mean depth = 149 m) than the other Great Lakes, but the EZD:SD ratio is about 0.29, which is slightly higher than the mean ratio for Lakes Michigan and Ontario but lower than the ratio for Lake Huron. The potential limiting factor(s) for each lake can vary depending on EZD:SD ratios, with Lakes Michigan, Ontario, and Superior more likely to be light limited than Lakes Erie and Huron.

Despite large differences in geographic position among stations, growth rate parameters were very similar among stations. Maximum growth rates and growth efficiency (α) were not significantly different among any of the lakes ($p < 0.05$). Thus, constraints on growth rates may be similar among all the lakes, and experimental evidence from one lake, e.g., Lake Ontario, may be applicable to all the lakes during the spring period. Moreover, similarity in growth rates across all of these stations may be used to suggest that regional climatic factors such as light and temperature provided important constraints on growth rates. While these factors are likely important, nutrient concentrations and food

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web structure are also similar across these lakes during spring mixing (Fahnenstiel et al. 1998). Total phosphorus concentrations ranged from 0.1 to 0.2 nM. Food web characteristics, i.e., autotrophic to heterotrophic, picoplankton to nannoplankton and microplankton ratios, etc., were also similar among lakes (Fahnenstiel et al. 1998). Thus, given the similarity of primary controlling factors, it is not surprising that growth rates are similar across all of the lakes.

Phytoplankton growth in the Laurentian Great Lakes may often be controlled by a complex interaction of light, nutrients, and possibly even temperature. All three of these environmental factors can be or are suboptimal in all of the Great Lakes during the spring isothermal mixing period. During spring mixing, both light availability and nutrient availability in the lakes are often near the threshold values for both nutrient and light limitation; neither light nor nutrients are in abundant supply. Soluble phosphorus concentrations during this period were typically <50 nM (G.L. Fahnenstiel, unpublished data). Moreover, the low temperatures (1–4°C) found at this time of year are often suboptimal for growth (Stoermer and Ladewski 1976; Reynolds 1984). Historically, light has been suggested as limiting phytoplankton growth in the Great Lakes during spring isothermal mixing (Nalewajko and Voltolina 1986; Scavia and Fahnenstiel 1987; Millard et al. 1996). The important role that light plays in controlling phytoplankton photosynthesis and growth was evident in our study. In situ light conditions were suboptimal for photosynthesis and growth during many of the sampling periods. Only in Lake Huron were ambient light levels consistently higher than threshold values for photosynthesis. However, the potential that other factors may be important in controlling phytoplankton growth during this period is a relatively new idea.

Another important factor controlling phytoplankton growth during spring isothermal mixing is nutrient availability. In Lake Ontario, which had the lowest EZD:SD ratios and thus the most potential for strict light limitation, nutrient additions at simulated in situ irradiances produced significant increases in phytoplankton growth rates and biomass. These results, combined with observation of light saturation at in situ irradiances, suggest that nutrients can limit phytoplankton growth. This nutrient role is consistent with earlier work in the upper Great Lakes (Superior and Michigan). Previous work has documented that phytoplankton from the upper Great Lakes exhibit low to moderate phosphorus deficiency during spring isothermal mixing (Nalewajko and Voltolina 1986; G.L. Fahnenstiel, unpublished data). This lack of strong phosphorus, or nutrient, limitation is consistent with our nutrient enrichment experiments, in which only a slight increase in growth rate occurred with nutrient additions. Only in the lower Great Lakes (Ontario and Erie) has no evidence of nutrient limitation been found during spring isothermal mixing (Lean et al. 1983, 1987; Millard et al. 1996).

Although nutrients may be important in controlling phytoplankton growth rates, they likely do not act alone but rather in combination with light and possibly even temperature. Evidence for this conclusion is based on several factors, including direct experimental evidence from the growth and nutrient enrichment experiments. In most instances, neither light nor nutrient availability alone constrained growth rates, for if either limitation was alleviated, only slight increases in growth rate were found, and rates remained relatively low. In situ growth rates were low, ranging from 0.04 to 0.15·day^{-1}, and the mean light-saturated rate μ_{max} was only 0.12·day^{-1}. Thus, under light-saturating conditions, growth rates would still be <0.2·day^{-1}. Similarly, alleviating nutrient limitation by adding a saturating nutrient concentration (0.5 µmol additions of phosphorus usually produce V_{max} in the Great Lakes; G.L. Fahnenstiel, unpublished data), as was done in nutrient enrichment experiments, increased growth rates by only 30–50%, leaving them still <0.3·day^{-1} in most cases. However, when both nutrient availability and light availability were increased, the highest growth rates (about 0.3·day^{-1}) and final biomass were found (12% light level with nutrient addition), suggesting that nutrients and light interact in controlling in situ rates. Because growth rates were only 0.3·day^{-1} when both light and nutrients were saturating, suboptimal temperatures (1–4°C) likely constrain maximum growth rates and possibly even in situ rates. Temperature has been thought to limit the maximum growth rate, whereas other factors (e.g., nutrients and light) were considered more important in controlling in situ rates (Epply 1972). However, at suboptimal temperatures during spring mixing, temperature may also interact with nutrient availability and constrain in situ growth rates. Under nutrient-limited conditions, suboptimal temperatures interact with nutrients and light in a complex manner and likely aggravate nutrient limitation (Rhee 1982). Also, temperature influences light requirements for growth (Morgan and Kalff 1979).

The likelihood that an interaction of light and nutrients controls phytoplankton growth in the Great Lakes during spring isothermal mixing is supported by a comparison of previous work documenting nutrient–light interactions and present conditions in the Great Lakes. Several authors have used laboratory cultures to evaluate interactions of nutrients and light in controlling growth rates when light and temperature are at suboptimal levels (Rhee and Gotham 1981; Fahnenstiel et al. 1984; Healey 1985). The combined effects of nutrient and light limitation are greater than the sum of individual effects, and at suboptimal irradiances, nutrients and light can compensate for each other in maintaining a given growth rate (Rhee and Gotham 1981). Rhee (1982) suggested that this compensatory relationship would make it difficult to separate the effects of light and nutrient limitation in the euphotic zone. Healy (1985) noted that over a narrow range of low irradiances, simultaneous limitation of light and nutrients can be found. Using laboratory cultures from the deep chlorophyll layer in Lake Michigan, Fahnenstiel et al. (1984) described a similar nutrient–light interaction, where the maximum steady-state yield was determined by the combined effects of nutrients and light. These nutrient–light interactions occurred at irradiances ranging from 8 to 78 µmol quanta·m^{-2}·s^{-1}. For unialgal cultures of *Synechococcus*, Healy (1985) reported interactions between 10 and 20 µmol quanta·m^{-2}·s^{-1}, and for cultures of *Scenedesmus*, Rhee and Gotham (1981) reported interactions between 7 and 17 W·m^{-2} or approximately 32–78 µmol quanta·m^{-2}·s^{-1} (J. Cullen, Dalhousie University, Halifax, N.S., personal communication). For natural assemblages of Lake Michigan phytoplankton, Fahnenstiel et al. (1984) found interactions to occur between 8 and 57 µmol quanta·m^{-2}·s^{-1}. These values for laboratory cultures are simi-

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lar to mean water column irradiances that exist in the Great Lakes during spring isothermal mixing. Mean water column irradiances in the Great Lakes ranged from 11 to 101 μmol quanta·m^{-2}·s^{-1} with a mean of 36 μmol quanta·m^{-2}·s^{-1}. Thus, nutrient–light interactions should occur in the Great Lakes during spring isothermal mixing.

The previously noted absence of nutrient limitation in Lakes Erie and Ontario during the spring period (Lean et al. 1983, 1987) and the evidence for strict light limitation during this period (Millard et al. 1996) may seem contradictory to our conclusions, but this apparent contradiction is easily explained. First, and most important, are the dramatic changes in the light climate that have occurred in the lower Great Lakes during the past two decades due to phosphorus load reductions and the impact of nonindigenous mussels (e.g., Holland 1993; Fahnenstiel et al. 1998). These large increases in light penetration would decrease the potential for light limitation and increase the potential for nutrient limitation and nutrient–light interactions. In the early 1970s, Thomson et al. (1974) reported k_{PAR} values from the spring mixing period of 0.57 and 0.34·m^{-1} in Lakes Erie and Ontario, respectively. In the late 1980s, k_{PAR} values from two stations in Lake Ontario during the April–May period were 0.26 and 0.32·m^{-1} (Millard et al. 1996). In 1993–1995, we reported a mean k_{PAR} value for stations in Lake Ontario of only 0.18·m^{-1}. This large increase in transparency in the 1990s produced an increase in the mean water column irradiance in Lake Ontario of 43–76% from the late 1980s and an 87% increase from the early 1970s. Assuming a k_{PAR} value of 0.29·m^{-1} for the late 1980s and 0.34·m^{-1} for the early 1970s (and similar physiological characteristics for phytoplankton communities), the increase in transparency in Lake Ontario would change the ratio $I_{k,g}$:$\bar{I}_{wc}$ from 0.6 in the early to mid-1990s to 1.0 in the late 1980s and to 1.1 in the early 1970s, respectively. Moreover, for photosynthesis, the ratio I_k:$\bar{I}_{wcd}$ would change from 1.3 in the 1990s to 2.2 in the 1980s and to 2.6 in the 1970s. Thus, at the time that Lean et al. (1983, 1987) and Millard et al. (1996) performed their experiments, the phytoplankton communities were likely light limited.

A second factor that needs consideration when comparing our results to those of Millard et al. (1996) are the differences between the photosynthetic and growth light saturation parameters (I_k versus $I_{k,g}$). Photosynthesis–irradiance relationships can be very different from growth–irradiance relationships, and it is not uncommon for growth to saturate at much lower irradiances than photosynthesis (Beardall and Morris 1976; Morris and Glover 1981). We reported a saturation irradiance for growth of 0.07 mol quanta·m^{-2}·h^{-1} (calculated for photoperiod only), whereas the threshold for photosynthesis was 0.16 mol quanta·m^{-2}·h^{-1}. These differences are important not only for the Great Lakes but also for all studies where photosynthesis–irradiance parameters are extrapolated to growth. The relationship between growth and photosynthesis will not be similar for all communities (Cullen 1990), and thus, extrapolating photosynthesis–irradiance parameters may produce erroneous conclusions.

Finally, nutrients appear to limit the maximum spring phytoplankton biomass across the Great Lakes. Carbon to chlorophyll ratios of spring phytoplankton and in situ chlorophyll concentrations suggest that light is not likely limiting biomass during late April – May (Parker et al. 1977; Fahnenstiel and Scavia 1987). However, prior to the development of this phytoplankton maximum, environmental variables that limit growth not only control the rate of biomass increase but potentially also biomass. In this study, we demonstrated that important rate processes such as growth and photosynthesis were controlled by nutrients and light. Thus, these same two factors are likely important factors controlling in situ biomass prior to the spring maximum. Temperature, acting through an interaction with nutrients, may also be important but clearly sets a range within which nutrient availability and light availability provide more proximate control.

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Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract: Neurotoxic pesticides are known to contaminate surface waters that provide habitat for salmonids, including some listed for protection under the U.S. Endangered Species Act. Despite their widespread use, the impacts of these pesticides on the neurological health of wild salmon are not well understood. Of particular concern are the organophosphate and carbamate insecticides that block synaptic transmission by inhibiting neuronal acetylcholinesterase. Here we assess the effects of diazinon, an organophosphate insecticide, on alarm pheromone induced antipredator responses and homing behavior in chinook salmon (*Oncorhynchus tshawytscha*). Nominal exposure concentrations (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$) were chosen to emulate diazinon pulses in the natural environment. In the antipredator study, diazinon had no effect on swimming behavior or visually guided food capture. However, the pesticide significantly inhibited olfactory-mediated alarm responses at concentrations as low as 1.0 $\mu g \cdot L^{-1}$. Similarly, homing behavior was impaired at 10.0 $\mu g \cdot L^{-1}$. Our results suggest that olfactory-mediated behaviors are sensitive to anticholinesterase neurotoxicity in salmonids and that short-term, sublethal exposures to these insecticides may cause significant behavioral deficits. Such deficits may have negative consequences for survival and reproductive success in these fish.

Résumé : On sait que les pesticides neurotoxiques contaminent les eaux de surface qui constituent l'habitat des salmonidés, notamment de certains poissons désignés comme protégés par la loi américaine sur les espèces en danger («Endangered Species Act»). Malgré leur usage répandu, on ne connaît pas bien les impacts de ces pesticides sur la santé neurologique des saumons sauvages. On s'inquiète particulièrement des effets des insecticides à base d'organophosphate et de carbamate, qui bloquent le transfert synaptique en inhibant l'acétylcholinesterase (AchE) dans les neurones. Nous évaluons ici les effets du diazinon, un insecticide organophosphaté, sur les réactions antiprédateur induites par les phéromones d'alarme et sur le comportement de retour chez le saumon quinnat (*Oncorhynchus tshawytscha*). Nous avons choisi des concentrations nominales d'exposition (0,1, 1,0 et 10,0 $\mu g \cdot L^{-1}$) pour simuler les administrations de diazinon dans le milieu naturel. Dans l'étude sur le comportement antiprédateur, le diazinon n'avait aucun effet sur le comportement de nage ni sur la capture de nourriture à vue. Toutefois, le pesticide inhibait de façon significative les réponses d'alarme médiées par l'olfaction à des concentrations de 1,0 $\mu g \cdot L^{-1}$. De même, le comportement de retour était troublé à 10,0 $\mu g \cdot L^{-1}$. Nos résultats permettent de penser que les comportements médiés par l'olfaction sont sensibles à la neurotoxicité anticholinesterase chez les salmonidés, et que des expositions sublétales de courte durée à ces insecticides peuvent causer d'importants déficits comportementaux, déficits qui peuvent avoir des conséquences négatives pour la survie et le succès de reproduction chez ces poissons.

[Traduit par la Rédaction]

Introduction

Wild Pacific salmon (*Oncorhynchus* sp.) populations are declining throughout the western United States (e.g., Myers et al. 1998). For example, several salmon stocks (defined as evolutionarily significant units) in the states of California, Oregon, Washington, and Idaho have recently been listed for protection under the U.S. Endangered Species Act (ESA). A significant factor in these declines is the deterioration or loss of freshwater habitat (National Research Council 1996). Land-use activities (e.g., forestry, agriculture, grazing, mining, and urban and industrial development) have complex

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and mostly negative consequences for salmon productivity. The effects of physical disturbances such as diking, erosion, channelization, sedimentation, loss of riparian vegetation, and loss of large woody debris on salmon productivity are reasonably well known (National Research Council 1996). However, many western river systems also receive substantial inputs of toxic chemicals, and the impacts of these compounds on salmon survival and reproductive health are poorly understood.

Rivers and streams in the western United States are routinely contaminated by a diverse mixture of insecticides, herbicides, and other biocidal compounds. Recent monitoring studies by the U.S. Geological Survey found 49 different pesticides in the surface waters of California's Central Valley (Dubrovsky et al. 1998), 50 in Oregon's Willamette basin (Wentz et al. 1998), and 23 in urban streams in the Puget Sound basin (U.S. Geological Survey 1999). Although these compounds are prevalent in streams and rivers that provide habitat for ESA-listed salmonids, the effects of individual pesticides and pesticide mixtures on salmon health are largely unknown. Consequently, there is considerable scientific uncertainty with respect to what role, if any, pesticides play in the decline of wild populations.

In general, the organophosphate and carbamate classes of insecticides are more acutely lethal to fish than other pesticides (EXTOXNET; pesticide information available at http://ace.orst.edu/info/extoxnet/). These compounds are neurotoxins that inhibit the activity of acetylcholinesterase (AChE), an enzyme that terminates acetylcholine-mediated, or cholinergic, neurotransmission. The enzyme is widely expressed throughout the fish nervous system (Weiss 1958; Ferenczy et al. 1997; Sturm et al. 1999). Correspondingly, AChE inhibition can lead to a distributed loss of nervous system function. At high concentrations, exposures to anticholinesterase neurotoxins produce spasms, paralysis, and eventual death (Post and Schroeder 1971). Organophosphates and carbamates frequently contaminate western rivers; for example, they account for 12 of 19 insecticides detected in the surface waters of California's Central Valley (Dubrovsky et al. 1998).

Diazinon, an organophosphate insecticide, is commonly detected in the aquatic environment (e.g., Dubrovsky et al. 1998). Diazinon is transported into rivers largely via stormwater runoff (Domagalski 1996), with rain events producing pesticide pulses in rivers and streams. These pulses are typically narrow and well defined, lasting from a few days to weeks (Kuivila and Foe 1995). While in-stream concentrations of diazinon have been measured as high as 36.8 $\mu g \cdot L^{-1}$ (Menconi and Cox 1994), they are more commonly less than 10.0 $\mu g \cdot L^{-1}$. A typical pulse of diazinon lasting for only a few days at concentrations of less than 10.0 $\mu g \cdot L^{-1}$ is unlikely to kill fish outright. The acute toxicity (96-h LC_{50}) of diazinon has not been determined for chinook salmon (*Oncorhynchus tshawytscha*), but the value is probably similar to the known LC_{50} values for congeneric rainbow trout (*Oncorhynchus mykiss*) (839 $\mu g \cdot L^{-1}$) or cutthroat trout (*Oncorhynchus clarki*) (2620 $\mu g \cdot L^{-1}$) (Novartis Crop Protection, Inc. 1997). If so, there is a substantial (approximately 2–3 orders of magnitude) difference between concentrations of diazinon commonly found in the environment and those required to produce acute lethality in the laboratory. Chronic toxicity (e.g., reduced egg production) has been reported in sheepshead minnow (*Cyprinodon variegatus*) at diazinon concentrations as low as 0.47 $\mu g \cdot L^{-1}$ (Goodman et al. 1979). However, chronic exposures can last for 1 or more months, which rarely, if ever, occurs under natural conditions. Thus, based on the results of conventional acute and chronic toxicity assays, it has been concluded that Pacific salmon are not at risk from the direct effects of diazinon in freshwater habitat (Novartis Crop Protection, Inc. 1997).

However, the conventional toxicity studies on which that conclusion was based were not explicitly designed to measure nervous system function. For example, conventional assays do not consider potential injury to the fish olfactory system despite the fact that sensory neurons are directly exposed to dissolved toxicants in the water. In fish, the detection of chemical cues (signal transduction) occurs within the olfactory rosettes, which are the principal structures in the fish olfactory organ. Olfactory receptor neurons, which bind odorant molecules, are embedded within a sensory epithelium that lines the folded lamellae of the rosettes (Zeiske et al. 1992). Chemosensory perception can be altered when dissolved neurotoxins either (*i*) compete with natural odorants for binding sites on membrane-associated receptor proteins, (*ii*) modulate the activation properties of these receptor proteins, or (*iii*) are translocated to the cytosol of the sensory neuron where they modify intracellular signalling events (Klaprat et al. 1992). The olfactory nervous system is sensitive to a wide range of dissolved contaminants (Sutterlin 1974; Brown et al. 1982; Klaprat et al. 1992), and a loss of olfactory capacity could interfere with many important behaviors in fish, including feeding, defense, schooling, migration, and reproduction (Kleerekoper 1969).

Recently, Moore and Waring (1996) found that environmentally relevant exposures to diazinon can desynchronize the reproductive physiology of prespawning Atlantic salmon (*Salmo salar*) by inhibiting the male's perception of a priming pheromone. Exposures to carbofuran, another anticholinesterase pesticide, resulted in a similar loss of function (Waring and Moore 1997). Sublethal exposures to carbofuran have also been shown to inhibit AChE enzymatic activity in the olfactory rosettes and the olfactory bulb of the common carp (*Cyprinus carpio*) (Haubruge and Toutant 1997). Collectively, these studies suggest that AChE plays an essential role in signal transduction within the olfactory epithelium. In addition, AChE inhibition may interfere with the normal integration of chemical cues within the olfactory forebrain.

Because chinook salmon rely on a wide range of olfactory-mediated behaviors for survival and reproductive success, a loss of olfactory capacity, while subtle, could have important negative consequences for the health of individual fish. In addition, olfactory cues provide the basis for imprinting and homing, which are critical for maintaining the integrity of wild populations (Dittman and Quinn 1996). Here, we use specific behavioral assays to determine whether diazinon disrupts olfactory nervous system function in chinook salmon. We show that sublethal, environmentally relevant exposures are sufficient to impair both alarm pheromone induced antipredator behavior and homing behavior in juvenile

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and adult animals, respectively. These findings are discussed in the context of freshwater habitat quality and the management of ESA-listed species in western river systems.

Materials and methods

Antipredator study

Chemical alarm signal induced antipredator behaviors were measured using an experimental design modified from Berejikian et al. (1999). Eyed chinook salmon eggs were obtained from the Minter Creek Hatchery, Pierce County, Wash. The eggs were incubated and hatched in a model stream at the National Marine Fisheries Service Manchester Experimental Station (Manchester, Wash.). The fish were reared in a circular tank (1.2 m in diameter) on a 16 h light : 8 h dark cycle and fed a standard commercial salmon diet several times daily, 5 days per week. A stock conspecific skin extract (alarm stimulus) was prepared by homogenizing approximately 100 cm^2 of skin from 23 fish in 1 L of deionized water. The extract was filtered twice through a polyester filter floss, aliquoted, and stored at –20°C.

Behavorial trials were carried out indoors in ten 170-L aquaria positioned within two nearly identical flumes (9.0 m long by 1.5 m wide; see Berejikian et al. (1999) for a complete description of tank design). In brief, each aquarium was constructed of opaque glass on three sides. The tanks were lined with gravel, and uniform overhead lighting was provided by a solid bank of wide-spectrum fluorescent lights. A 15-cm^2 tile was situated approximately 5 cm above the gravel to provide overhead cover, and a 2.5-cm-high plastic ring was placed around the tile to add lateral cover. The water used during the maintenance, exposure, and testing of fish was gravity-fed from an on-site well. Water temperatures ranged between 10 and 14°C, and chemical properties were pH 8.0, total hardness (as $CaCO_3$) 65 $mg \cdot L^{-1}$, conductivity 150 $\mu mho \cdot cm^{-1}$, sodium 5.2 $mg \cdot L^{-1}$; magnesium 7.3 $mg \cdot L^{-1}$, and calcium 14 $mg \cdot L^{-1}$. Water was introduced at a rate of 6 $L \cdot min^{-1}$ via a polyvinyl tube that terminated middepth on one side of the aquarium, and depth was maintained at 25 cm by a double siphon on the opposite side of the tank.

It has been previously shown in Atlantic salmon parr that a brief exposure to diazinon (30 min at 1 $\mu g \cdot L^{-1}$) is sufficient to reduce the responsiveness of the olfactory epithelium to an odor stimulus. In addition, the olfactory epithelium does not recover within the first few hours postexposure (Moore and Waring 1996). Based on these findings, we chose to expose chinook salmon parr to diazinon for 2 h and allow them to recover for 1 h before initiating the antipredator behavioral trials. Diazinon exposures were conducted in 20-L opaque glass aquaria. Exposure concentrations (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$) were prepared fresh each day by dissolving an analytical-grade stock of diazinon (Chem Services) in a small volume of acetone (0.01% final concentration). The diazinon–acetone mixtures were then diluted into 10 L of well water. Acetone alone (0.01%) was used as a vehicle control.

Diazinon exposures and behavioral testing were conducted in the following sequence. On the day prior to testing, a single juvenile chinook salmon was placed in each of the 170-L aquaria. The fish were allowed to acclimate for at least 24 h, during which they were fed a single meal (1.5% of body weight). On the day of the trial, individual fish were removed from their tanks and transferred to separate 20-L exposure aquaria that had been previously treated with diazinon or acetone as a vehicle control. After a 2-h exposure, the fish was returned to the 170-L test aquarium and allowed to reacclimate for 1 h. Subsequently, approximately 3 mL of live *Daphnia* (collected fresh from a local pond) was introduced into the tank via the inflow tube. After a 2-min interval, prestimulus feeding behavior was recorded for 8 min. The conspecific skin extract (100 µL) was then added through the water inflow tube. After a 10-s delay, antipredator behaviors were recorded during an 8-min poststimulus interval.

Prestimulus feeding behaviors and the poststimulus antipredator response were recorded using behavioral data acquisition software (Observer 2.0). Specifically, we measured the frequency of food strikes, the amount of time spent motionless, and the amount of time spent in the lower, middle, and upper third of the water column. Fish were defined as motionless if they remained stationary (moved less than 1 cm) for 3 s. During the alarm response, many animals maintained a stationary position in the water column by rapidly fanning their pectoral fins. The frequency of darting behavior (burst swimming) and amount of time spent under cover were also measured; however, as reported previously (Berejikian et al. 1999), these behaviors were infrequent and we did not consider them significant antipredator behaviors.

All trials were conducted blind, i.e., the observer had no knowledge of the treatment conditions. Overall, 17–20 individual juvenile chinook salmon were tested at each exposure concentration for a total of 75 fish (fork lengths between 4.7 and 6.3 cm, mean = 5.5 cm). Each fish was used only once, and the relationship between diazinon treatment and test aquarium was randomized between trials. In preliminary experiments, three control fish were exposed to a higher concentration of skin extract. The poststimulus behaviors of these animals were not included in the analysis.

Homing study

Homing behavior has previously been used to measure olfactory capacity in chinook salmon (Quinn et al. 1988). In the present study, a total of 160 maturing chinook salmon were collected from the University of Washington hatchery between October 9 and November 10, 1998. The pond was seined twice weekly, and returning males (age 1) were captured and transferred to a concrete raceway. The fish (fork lengths between 14.8 and 30.1 cm, mean = 23.7 cm) were in good condition, without lacerations or obvious signs of disease.

After each day of seining, the fish captured were immediately divided into four treatment groups and fin-clipped for identification. The following day, static 24-h exposures to diazinon (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$ nominal concentrations) or a vehicle control (acetone, final concentration <0.01%) were conducted in four 800-L tanks filled with lake water. Diazinon dilutions were prepared fresh each day from an analytical-grade stock solution (Chem Services). Each tank was fitted with a Living Stream® chiller to maintain aeration and a constant water temperature (13–14°C).

Following exposures, fish were transferred to a 775-L stainless steel tank and transported to a freshwater release site approximately 2 km downstream from the hatchery. A total of 160 fish (40 per treatment group) were treated and released during the course of the experiment. Homing fish (i.e., those returning to the hatchery) were identified during subsequent seining operations at the hatchery that lasted until November 23. In addition, a total of 20 fish (five per treatment group) were placed together in a concrete raceway at the hatchery. These animals were in the same size range as the fish that were treated and released. They were monitored throughout the study to ensure that the diazinon exposures did not grossly reduce the longevity of animals used in the homing experiment.

Results

Antipredator study

A 2-h diazinon exposure at nominal concentrations of 0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$ had no significant effect on either basal foraging behavior or swimming activity (Fig. 1). In general, individual fish fed actively throughout the 8-min prestimulus

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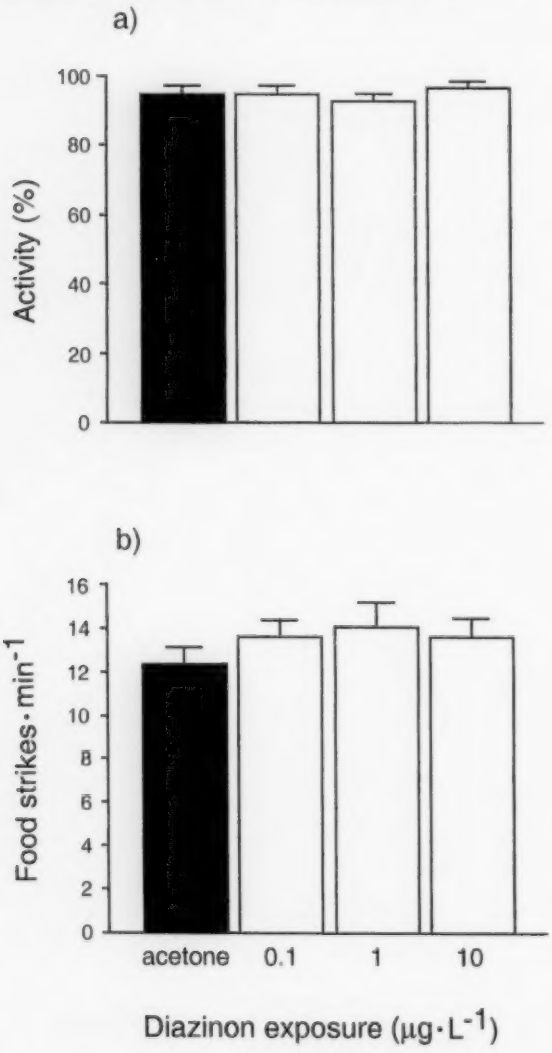
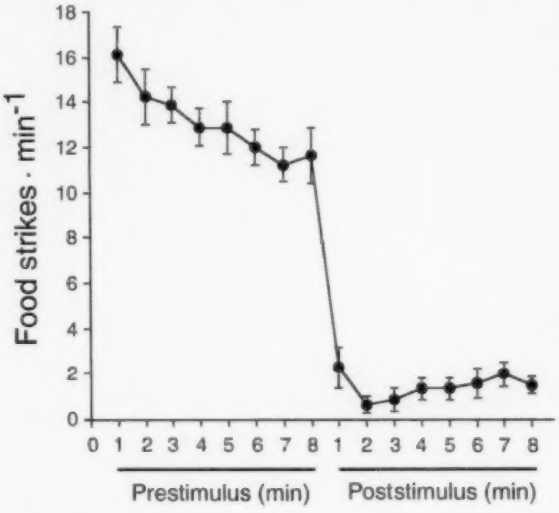
Fig. 1. Short-term diazinon exposures have no effect on (*a*) swimming activity or (*b*) feeding behavior in chinook salmon parr. Individual fish were removed from their aquaria and exposed to diazinon (0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$ nominal concentrations) or a vehicle control (acetone) for 2 h. The fish were subsequently returned to their aquaria and allowed to reacclimate for 1 h. Food (live *Daphnia*) was added to the tank and the behavior of each fish was recorded (see Materials and methods) for 8 min. Values represent the mean ± SEM for 20 animals. Each animal was tested only once. There were no significant differences among treatment groups ($p > 0.05$, Fisher's test).



interval (Fig. 2). As previously reported (Berejikian et al. 1999), juvenile chinook salmon show stereotypic antipredator behaviors in response to a chemical alarm signal. Qualitatively, animals moved towards the bottom of the tank and became motionless when a conspecific skin extract was added to the tank. In addition, control fish abruptly stopped feeding in response to the skin extract (Fig. 2). The alarm

Fig. 2. Conspecific skin extract elicits an abrupt reduction in feeding behavior in control chinook salmon. Feeding activity was recorded for 8 min before (prestimulus) and after (poststimulus) the addition of the skin extract to the aquaria. Values are data from 1-min intervals and represent the mean ± SEM for 17 animals.



signal elicited a significant decrease in both swimming activity ($p < 0.001$, Fisher's test; Fig. 3*a*) and feeding behavior ($p < 0.001$, Fisher's test; Fig. 3*b*) in controls. The fish also tended to move to the lower third of the water column. However, several fish were already foraging in the lower part of the water column when the extract was introduced, so the pre- and post-stimulus differences in this measure were less pronounced.

Compared with control animals, diazinon-treated fish remained more active and fed more frequently when exposed to the conspecific skin extract (Fig. 4). Although some treated fish exhibited antipredator behaviors, many continued to forage in the presence of the alarm signal. The effect of diazinon on swimming and feeding behavior was significant at concentrations of 1 and 10.0 $\mu g \cdot L^{-1}$ ($p = 0.05$, Fisher's test), with the exception of the 10.0 $\mu g \cdot L^{-1}$ dose, which had a marginal effect ($p = 0.06$) on poststimulus swimming activity.

Homing study

Fewer diazinon-treated chinook salmon returned to the hatchery compared with control fish (Fig. 5). Overall returns were 40% (16 of 40) for control fish, 30% (12 of 40) for the 0.1 and 1.0 $\mu g \cdot L^{-1}$ exposure groups, and 15% (six of 40) for the 10.0 $\mu g \cdot L^{-1}$ exposure group. The effect of diazinon on homing success was significant at the 10.0 $\mu g \cdot L^{-1}$ exposure ($p < 0.01$, chi-square contingency analysis). Diazinon did not appear to have any gross effects on chinook salmon longevity. Compared with controls, there were no significant differences in the survival of diazinon-treated fish ($n = 5$ for each treatment group) that were held in raceways at the hatchery. Survival durations (mean ± SEM) for the different treatment groups were 26 ± 9 days for controls, 31 ± 11 days at 0.1 $\mu g \cdot L^{-1}$, 24 ± 13 days at 1.0 $\mu g \cdot L^{-1}$, and 25 ± 12 days at 10.0 $\mu g \cdot L^{-1}$.

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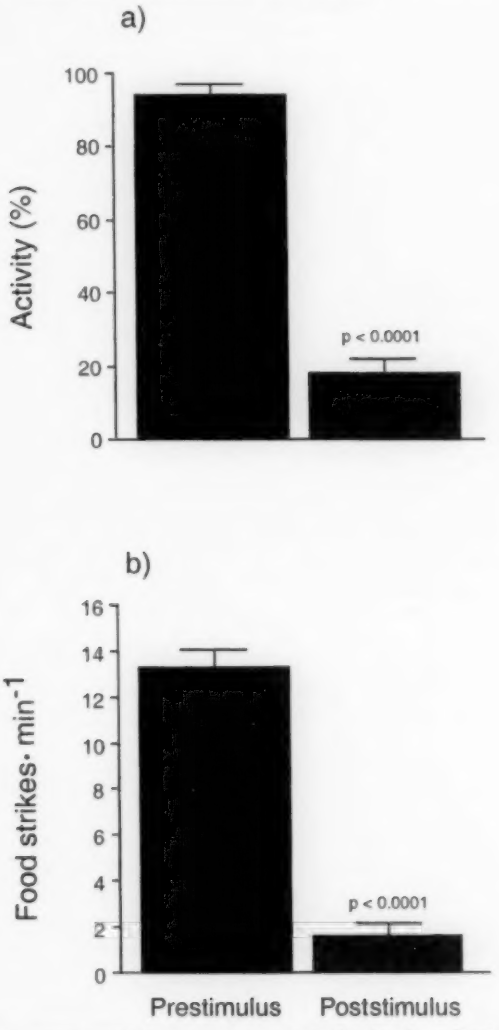
Fig. 3. Control chinook salmon significantly decrease both (*a*) swimming activity and (*b*) food capture behavior in response to the alarm stimulus ($p < 0.001$, Fisher's test). Values represent the mean ± SEM for 17 animals, and the data from an entire interval (prestimulus or poststimulus) are combined.

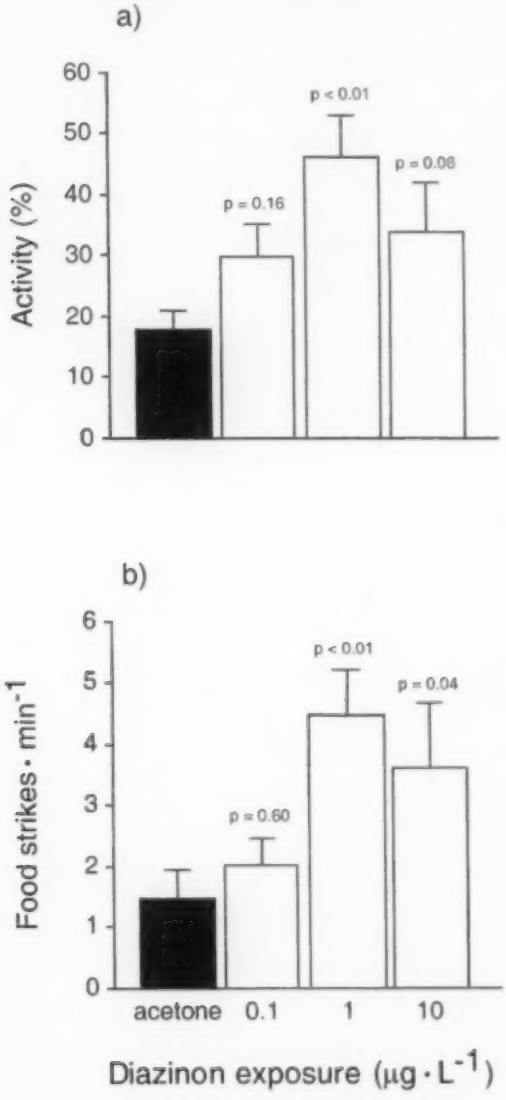


We were unable to test the hypothesis that diazinon-treated fish take longer to return to the University of Washington hatchery than controls. Returning fish were collected two or three times each week as part of normal hatchery operations. Consequently, we did not obtain precise temporal data on when fish from different treatment groups reentered the pond. Moreover, the small males used in this study occasionally avoided the net during seining. However, we found no significant differences in time to recapture (mean ± SEM) between control fish (11.8 ± 2.2 days) and animals exposed to diazinon at 0.1 $\mu g \cdot L^{-1}$ (11.0 ± 2.8 days), 1.0 $\mu g \cdot L^{-1}$ (11 ± 2.1 days), and 10.0 $\mu g \cdot L^{-1}$ (7.5 ± 3.0 days).

Discussion

Olfaction plays an important role in the complex life his-

Fig. 4. Antipredator behaviors are reduced in diazinon-exposed chinook salmon. Control fish responded to the conspecific skin extract by reducing their foraging activity and freezing, i.e., poststimulus fish were (*a*) largely inactive and (*b*) fed only infrequently (solid bar in Figs. 4*a* and 4*b*, respectively). The magnitude of the antipredator response was reduced in diazinon-exposed fish (2 h at 0.1, 1.0, and 10.0 $\mu g \cdot L^{-1}$), and they were (*a*) more active and (*b*) fed more often than controls. The effect of diazinon was significant at the 1.0 and 10.0 $\mu g \cdot L^{-1}$ exposures ($p = 0.05$, Fisher's test) with the exception of the marginal ($p = 0.06$) effect of 10.0 $\mu g \cdot L^{-1}$ on poststimulus swimming activity.



tories of Pacific salmon. We found that environmentally realistic exposures to the insecticide diazinon significantly disrupt olfactory-mediated behavior in chinook salmon. Our findings, together with recent studies of Atlantic salmon (Moore and Waring 1996, 1998; Waring and Moore 1997), demonstrate that the salmon olfactory nervous system is par-

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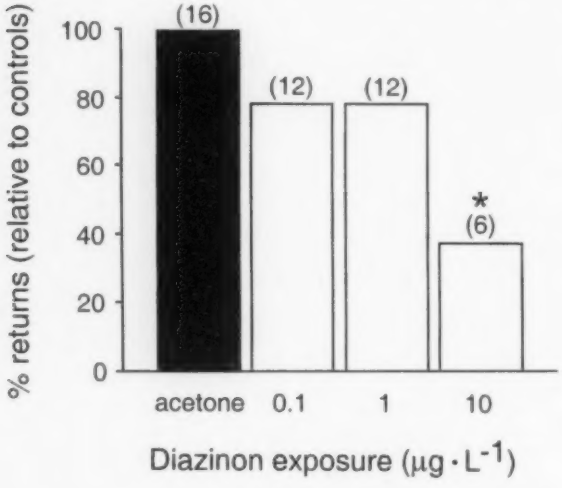
Fig. 5. A 24-h exposure to diazinon disrupts homing in chinook salmon males. Compared with controls, fewer diazinon-exposed fish returned to the University of Washington hatchery from a release site approximately 2 km downstream. A total of 40 fish were released in each treatment group. Numbers in parentheses indicate the total number of fish returning. The effect of diazinon was significant at the 10.0 $\mu g \cdot L^{-1}$ exposure ($p < 0.01$, chi-square contingency analysis).



ticularly sensitive to dissolved neurotoxins. Diazinon has now been shown, in salmonids, to impair reproductive priming (Moore and Waring 1996) as well as predator avoidance and homing behaviors (present study). The survival and reproductive success of wild Pacific salmon may, therefore, be compromised in river systems that are periodically contaminated with diazinon and other neurotoxic pesticides.

Antipredator behavior

Overall, the results of the antipredator experiment are consistent with the hypothesis that diazinon disrupts olfactory nervous system function in juvenile fish. Our findings confirm earlier studies (Brown and Smith 1997, 1998; Berejikian et al. 1999) demonstrating a chemical alarm system in salmonids. Moreover, when compared with controls, diazinon-exposed chinook salmon parr showed a reduced antipredator response to the conspecific skin extract (they were more active and fed more often in the presence of the alarm stimulus). Alarm behaviors reduce the likelihood that an animal will be preyed upon during an encounter with a predator (Smith 1992). For example, Mathis and Smith (1993) found that fathead minnows (*Pimephales promelas*) exposed to a conspecific alarm pheromone survived predation by a northern pike (*Esox lucius*) for approximately 40% longer than fathead minnows that were not exposed to the pheromone. Although not tested directly, the disruption of the alarm response by diazinon would likely increase the vulnerability of chinook salmon parr to predation.

In general, environmental toxins can reduce the ecological performance of a prey animal by adversely affecting its behavior (Mesa et al. 1994). In chinook salmon parr, we have shown that diazinon interferes with the early olfactory detection of a nearby predator, which is critical for escape and survival (Mesa et al. 1994). As a consequence, diazinon-exposed parr would be in a substandard condition at the time of a predatory interaction. For example, the alarm substance elicited subtle shifts in behavior during the poststimulus observation period. Individual fish would sometimes continue to search for food, but in a more restricted area, or they would continue to strike at food, but only if a *Daphnia* drifted near their stationary position. Presumably, the decision to forage in the presence of an alarm signal reflects a balance between each animal's appetitive or motivational state and its individual perception of predation risk. Decision-making, whereby fish balance the trade-off between food acquisition and the risk of mortality, is an important factor in predator–prey interactions (Mesa et al. 1994). For example, juvenile coho salmon (*Oncorhynchus kisutch*) restrict their foraging behavior following the visual detection of a predator (Dill and Fraser 1984; Martel and Dill 1993). In the present study, control fish sharply reduced their feeding activity in response to the alarm signal. By contrast, some diazinon-treated fish continued to forage at levels that were inappropriate for the situation, perhaps because they were unable to accurately gauge the potential of a predatory encounter.

The results from the antipredator study suggest that the olfactory nervous system is more sensitive to anticholinesterase neurotoxins when compared with the motor nervous system (e.g., the networks that underlie swimming behavior). Diazinon had no observed effect on swimming activity or visually guided food capture behavior at concentrations that significantly disrupted alarm signal evoked antipredator behaviors. Swimming performance is a common behavioral measure of sublethal anticholinesterase toxicity in salmonids and other fishes (e.g., Cripe et al. 1984; Little et al. 1990; Van Dolah et al. 1997). Although previous studies have reported anticholinesterase-induced swimming impairment, the thresholds for behavioral dysfunction were generally at concentrations or durations that exceed conditions in the natural environment. By these measures, it could be interpreted that anticholinesterases pose little risk for wild salmon. However, our results suggest that motor networks may be relatively insensitive to anticholinesterases. Consequently, toxicity thresholds derived from swimming assays may underestimate the susceptibility of the nervous system as a whole.

Homing

In the homing study, fewer diazinon-treated fish returned to the University of Washington hatchery compared with controls. Chinook salmon rely on olfactory cues to guide their upstream movements during the freshwater phase of their homeward migration (Hasler and Scholz 1983), and the weight of evidence suggests that diazinon-exposed fish were less able to smell and thus were less able to navigate back to their natal stream (Moore and Waring 1996). However, the results of the homing study should be viewed as preliminary for several reasons. First, the overall return of maturing males to the hatchery in the fall of 1998 was low, which limited the number of fish in each treatment group. Second, posttreatment returns were small in all four exposure groups. Only 40% of control fish returned to the hatchery, compared with approximately 80% in previous studies of homing in older salmon (e.g., Quinn et al. 1988). The reason for this

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discrepancy is not clear. It may reflect differences in the ages of the fish used or environmental conditions or both. Third, the hatchery pond was seined biweekly, and without precise temporal return data, we were unable to determine whether diazinon-treated fish took longer to home relative to controls. Finally, although our longevity experiment indicates that it is unlikely that diazinon exposures caused a delayed gross mortality, we cannot rule out the possibility that exposures may have subtly diminished postrelease survival. This could also account for the observed differences in returns. Nevertheless, significantly fewer fish returned to their natal stream to spawn after a short-term exposure to diazinon.

Dissolved neurotoxins may increase the incidence of straying among wild and hatchery-reared salmon that must traverse pesticide-contaminated rivers during their homeward migration. Straying, in which fish return to nonnatal streams to spawn, is an adaptive mechanism for colonizing new habitat and avoiding unfavorable local conditions (reviewed by Quinn 1993). However, straying of hatchery fish could compromise the genetic integrity of wild populations (Quinn 1993). Reciprocally, straying of wild salmon can diminish the number of animals remaining to spawn in a given stream. A loss of olfactory nervous system function would presumably lead to an increase in straying, since anosmic (nares-occluded) fish are unable to home (Wisby and Hasler 1954). Since sublethal exposures to pesticides and other contaminants can render fish functionally anosmic (reviewed by Klaprat et al. 1992), the presence of these toxicants in the freshwater environment could increase straying, thereby undermining the genetic integrity of ESA-listed species. This represents an important area for future research, especially since the factors that determine normal patterns of straying in wild and hatchery salmon are still poorly understood (Quinn 1993).

Management implications

Conventional chronic and acute toxicity studies, and the LC_{50} experimental paradigm in particular, may underestimate thresholds for neurobehavioral toxicity in salmonids. Consequently, the use of mortality data may not be appropriate for risk assessment in the context of the ESA. For example, our data and the study by Moore and Waring (1996) indicate that environmentally relevant exposures to diazinon can disrupt olfactory capacity in the context of survival and reproductive success, both of which are key management considerations under the ESA. These findings contrast with an ecological risk assessment of diazinon in the Sacramento and San Joaquin River basins (Novartis Crop Protection, Inc. 1997), which concluded that salmon are unlikely to be at risk from direct acute effects of diazinon residues in the water. Importantly, the latter study relied extensively on mortality (LC_{50}) measurements, an approach that has several drawbacks in the context of the ESA. First, to our knowledge, there have been no reports of diazinon-induced fish kills in western streams. Given this, acute mortality has little or no relevance to conditions in the natural environment. Second, LC_{50} studies are not explicitly designed to measure nervous system function. Consequently, it is impossible to accurately predict from LC_{50} values the exposures at which diazinon could cause sublethal behavioral disorders in wild salmon. Third, the LC_{50} values for diazinon in representative salmonids are approximately 2–3 orders of magnitude higher than the highest concentrations of pesticide that are commonly detected in the aquatic environment. This represents a perceived margin of safety that, when taken alone, may significantly underestimate the actual risk that ambient levels of diazinon and related pesticides pose for salmon.

Diazinon pulses are common in western rivers and streams. This insecticide was detected, at concentrations greater than 0.01 $\mu g \cdot L^{-1}$, in 71% of the surface water samples from California's San Joaquin and Tulare basins (Dubrovsky et al. 1998) and in 35% of the samples taken from Oregon's Willamette basin (Wentz et al. 1998). Similarly, diazinon was recently found at 100% of the sites sampled in an analysis of urban streams in Puget Sound, Washington State (U.S. Geological Survey 1999). Although in-stream pulses of diazinon alone may reach levels high enough to cause behavioral dysfunction in wild salmon, diazinon is only one of many current-use pesticides that cooccur in the aquatic environment. Organophosphates and carbamates both inhibit AChE (Millard and Broomfield 1995), and they are likely to have an additive impact on AChE-mediated functions in the fish nervous system. In the surface waters of California's Central Valley, the organophosphates azinphos-methyl, chlorpyrifos, diazinon, disulfoton, fonofos, malathion, methyl parathion, and terbufos and the carbamates aldicarb, carbaryl, carbofuran, and methomyl all cooccur (Dubrovsky et al. 1998). The additive effects of these pesticides on the salmon nervous system have not been studied, and it remains an important area for future research.

In conclusion, a properly functioning nervous system is critical for the expression of animal behavior. We have shown that environmentally relevant exposures to diazinon are sufficient to disrupt olfactory-mediated behaviors in chinook salmon. These behavioral deficits may, in turn, have negative consequences for the survival and reproductive success of animals under natural conditions. Since diazinon and other neurotoxic pesticides with a similar mode of action are known to contaminate river systems in the western United States that provide freshwater habitat for several ESA-listed species, they represent an unknown but potentially significant obstacle to salmon recovery efforts.

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Time series acoustic observations of macrozooplankton diel migration and associated pelagic fish abundance

Richard E. Thomson and Susan E. Allen

Abstract: In April 1997, we deployed an upward-looking 165-kHz water acoustic structure profiler (WASP) at a depth of 240 m near the head of Barkley Canyon on the west coast of Vancouver Island. Acoustic backscatter intensity, recorded every 10 s at 2-m intervals through the water column, revealed that zooplankton aggregated in well-defined layers near the canyon floor during daylight hours. Several hours prior to sunset, the animals began a slow vertical ascent, which ended 7.2 ± 4.2 min after sunset with a rapid ascent to the surface. Upward migration attained maximum ascent rates of 6.3 ± 0.5 cm·s^{-1} and ended 55.4 ± 2.3 min after sunset (18.6 ± 2.4 min before nautical twilight). Zooplankton remained near the ocean surface for several hours before beginning a slow descent, followed by a rapid-descent 24.6 ± 3.2 min before sunrise. Zooplankton quickly descended below major fish aggregations and ended their rapid descent 22.6 ± 8.7 min after sunrise, reaching the canyon floor 2–4 h after sunrise. Small fish targets were observed near the ocean surface during the night. Large fish targets generally did not arrive until dawn and were more concentrated at middepth.

Résumé : En avril 1997, nous avons déployé un profileur de structure acoustique (WASP) de 165 kHz à une profondeur de 240 m près du fond du canyon Barkley, sur la côte ouest de l'île de Vancouver. L'intensité de la rétrodiffusion acoustique, enregistrée toutes les 10 secondes à intervalles de 2 m dans toute la colonne d'eau, a révélé que le zooplancton se rassemblait en couches bien définies près du plancher du canyon pendant les heures de jour. Plusieurs heures avant le crépuscule, les animaux entamaient une lente montée verticale, qui se terminait 7,2 ± 4,2 min après le coucher du soleil par une rapide ascension jusqu'à la surface. Cette migration verticale atteignait une vitesse maximale de montée de 6,3 ± 0,5 cm·s^{-1}, et se terminait 55,4 ± 2,3 min après le coucher du soleil (18,6 ± 2,4 min avant le crépuscule nautique). Le zooplancton restait près de la surface de l'océan pendant plusieurs heures avant d'entamer une lente descente, suivie d'une descente rapide 24,6 ± 3,2 min avant le lever du soleil. Le zooplancton descendait rapidement au-dessous des grands rassemblements de poissons et terminait sa descente rapide 22,6 ± 8,7 min après le lever du soleil, atteignant le plancher du canyon deux à quatre heures après le lever du soleil. Des petites cibles de poissons ont été observées près de la surface de l'océan pendant la nuit. Les grosses cibles de poissons n'arrivaient généralement qu'à l'aube, et étaient plus concentrées à mi-profondeur.

[Traduit par la Rédaction]

Introduction

A primary goal of the Canadian GLOBEC program was to determine the role of submarine canyons off the west coast of Vancouver Island in enhancing biological productivity during times of upwelling-favorable winds. Observations (Freeland and Denman 1982; Hickey 1997; Vindeirinho 1998) and modeling studies (Wroblewski 1982; Allen 1996; Klinck 1996) indicate that up-canyon currents generated during periods of persistent upwelling winds result in increased onshore transport of nutrient-rich water from intermediate depths over the continental slope. The advection of nutrient-rich water toward the downstream end of the shoaling canyon head presumably leads to greater entrainment of nutrients into the euphotic zone and greater phytoplankton abundance over the shelf and slope. Enhanced phytoplankton abundance subsequently leads to increased zooplankton productivity (Mackas and Sefton 1982; Simard and Mackas 1989; Pillar et al. 1989). Productivity is expected to be especially high at the heads of submarine canyons, where deep upwelled canyon water is brought in close contact with the relatively shallow waters of the outer shelf, and in regions of strong shelf-break upwelling, where food density is high and advective displacement is minimized (Mackas et al. 1997).

Concurrent time series of plankton abundance and physical oceanic properties are needed to establish links between coastal productivity and wind-induced upwelling (Ware and Thomson 1991; Thomson and Ware 1996; Mackas et al. 1997). Because direct time series measurement of ocean pro-

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Fig. 1. Location of the WASP mooring at the head of Barkley Canyon on the continental margin of southwest Vancouver Island. Also shown are the ancillary current meter mooring sites (BC01, BC02, BC03, and A1) and the location of permanent weather buoy 46206. Water depths are in metres.

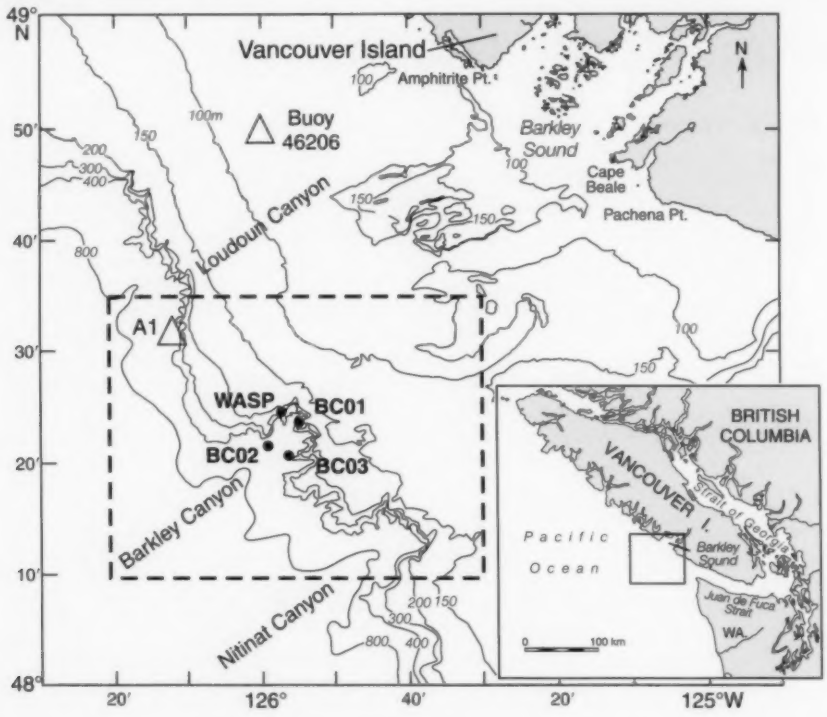
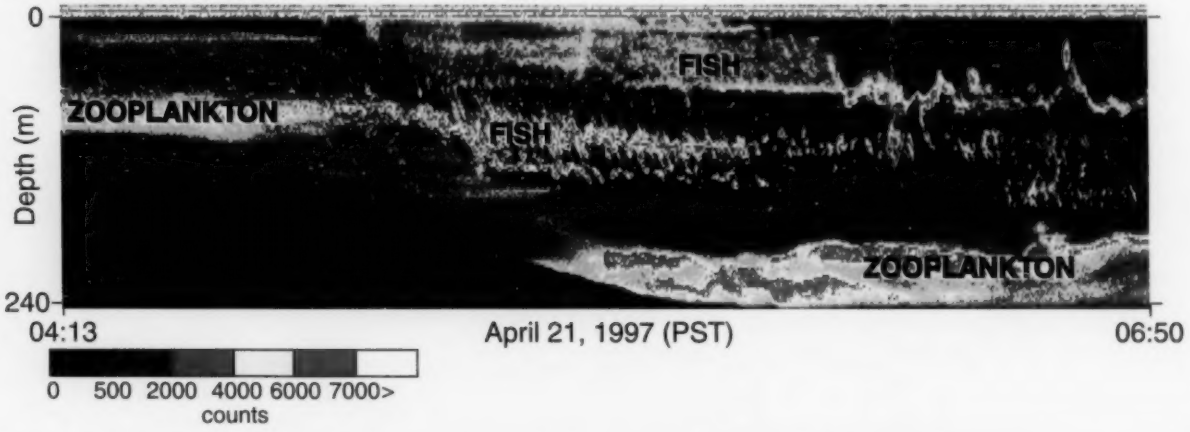


Fig. 2. Short segment of the "raw" acoustic backscatter intensity covering the 2.5-h early-morning period 04:13–06:50 PST on April 21, 1997 (Fig. 4 shows the entire daily record). Each acoustic pixel spans a time of 10 s and a depth range of 2 m. White denotes values in excess of 7000 counts, while black denotes values below 500 counts. Red denotes values of 6000–7000 counts and blue 500–700 counts. The ocean surface (240 m above bottom) is delineated by the base of the fuzzy red–white line at the top of the figure.



ductivity is both difficult and costly, more effort is being invested in remote sensing methods. Satellite-sensed ocean color is commonly used to estimate phytoplankton abundance during cloud-free periods (Thomas and Emery 1986; Denman and Abbott 1988; Thomas and Emery 1988), and shipboard acoustic sounders are used to map zooplankton abundance during oceanographic surveys (Simard and Mackas 1989; Mackas et al. 1997). Moored acoustic sensors (e.g., Rippeth and Simpson 1998) are a logical choice to examine long-term changes in zooplankton abundance but have been hampered by a lack of rapid (approximately 1 s) sampling devices with high data storage capabilities.

As part of our goal to link zooplankton biomass variability to the onset and persistence of coastal upwelling off Van-

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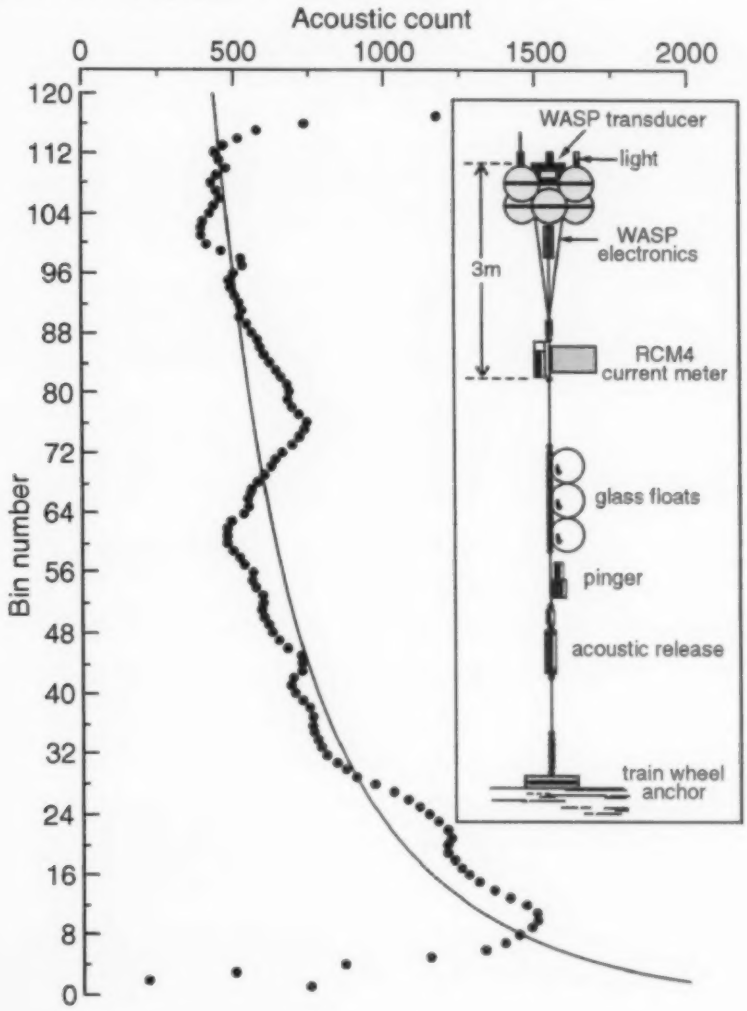
Fig. 3. Record mean calibration profile (dotted line) obtained by averaging the acoustic intensity time series for each depth bin. The profile is representative of the "background" acoustic backscatter intensity as a function of distance r from the WASP transducer. As indicated by the least-squares fit to the calibration data (solid line), the acoustic signal attenuates roughly exponentially with r. The inset presents a schematic of the mooring configuration.



couver Island, we deployed an upward-looking, single-beam 165-kHz water acoustic structure profiler (WASP) in 240 m of water at the head of Barkley Canyon in April 1997 (Fig. 1). The WASP recorded average acoustic backscatter intensity every 10 s at 2-m depth increments and was located within a wider array of current meter and thermistor string moorings that we maintained from April to October 1997. Although Barkley Canyon is one of the smaller canyons, it is thought to contribute significantly to the high productivity and fishery abundance of the region (Foreman and Thomson 1997; McFarlane et al. 1997). The WASP was moored at site BC04 at 15:16 local time (23:16 UTC) on April 18, 1997, but failed 5 days later because of a small leak through a damaged O-ring seal.

In this paper, we use the rapidly sampled, high vertical resolution acoustic data from the WASP to define the timing of macrozooplankton diel migration with respect to changing atmospheric light conditions and pelagic fish abundance. A time series of fish abundance is extracted from the acoustic data using a custom-designed notch filter. We also examine day-to-day variations in diel migration in relation to changing incoming solar radiation and nighttime cloud cover.

Materials and methods

The WASP, together with an Aanderaa RCM4 current meter, was moored on the canyon floor within a specially designed stainless steel frame supported by a glass float collar. In this configuration, the current meter (which measures water temperature and conductivity as well as current velocity) was at a nominal depth of 15 m above bottom and the upward-looking transducer 16 m above bottom. The WASP was programmed to collect approximately

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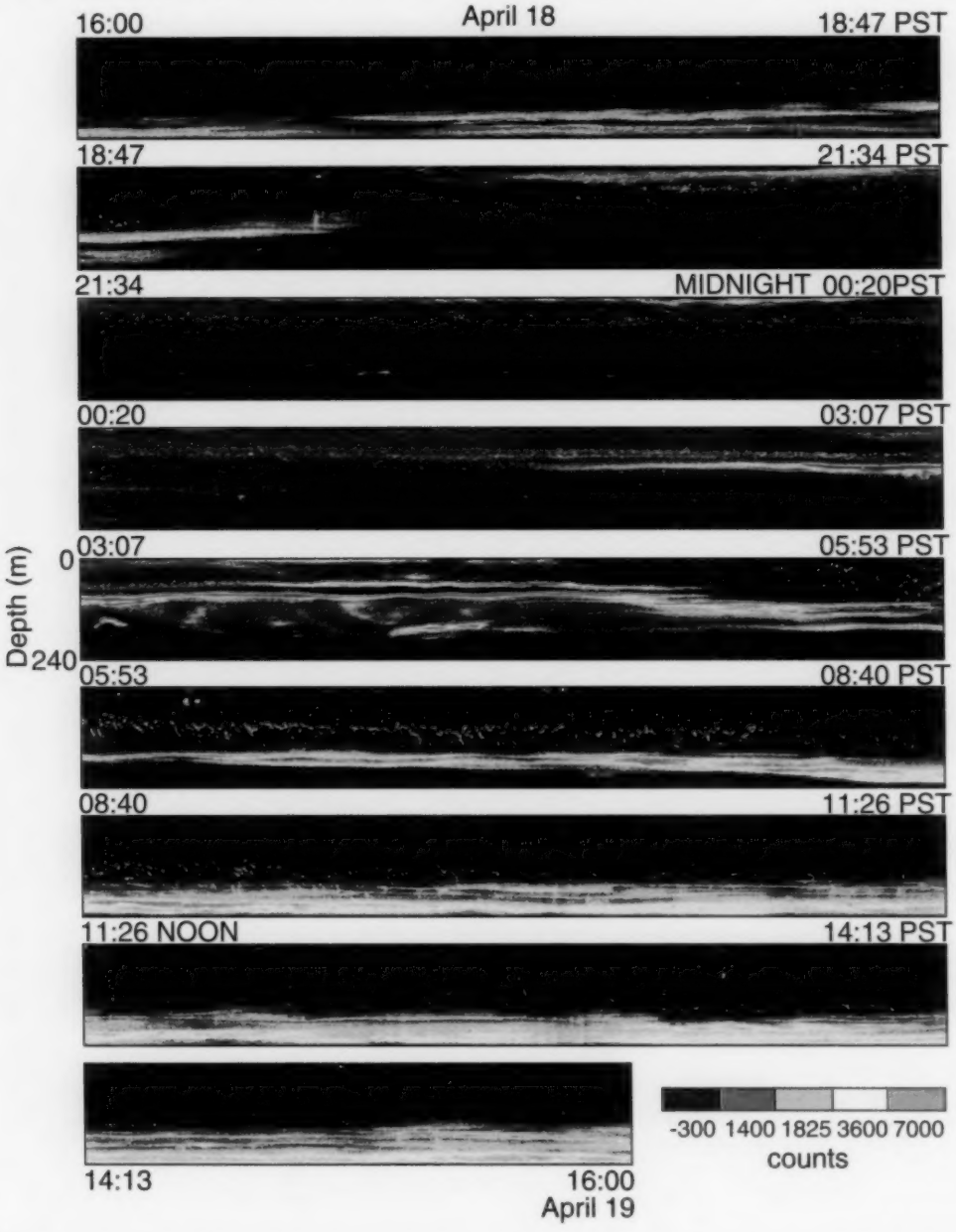
Fig. 4. Daily time series of acoustic backscatter intensity anomaly (mean profile removed) in Barkley Canyon covering the 1997 periods 16:00 PST on April 18 to 16:00 PST on April 19 and 16:00 PST on April 20 to 16:00 PST on April 21. Each acoustic pixel covers a duration of 10 s and a depth range of 2 m. Warmer colors denote higher values (see color bar).



6 months of data at bin lengths of 2 m over a maximum range of 125 bins. Based on chart datum of 240 m and the known tidal range of 4 m, the nominal sampling range of the WASP was 226 ± 2 m, and the relationships among bin number, d, height-above-bottom, H (metres), and the midbin depth, D (metres), are given by

$$(1a) \quad H(d) = 15 + 2d$$

$$(1b) \quad D(d) = 245 - H(d)$$

where $1 \leq d \leq 115$. With the exception of the first bin, which was highly distorted by acoustic reverberation, and the last bin, which was affected by side-lobe contamination, the instrument generated reliable acoustic data throughout the full water depth. (The beam angle of the transducer is 5.5° at the –3 dB level and the first side-lobe of the transducer is attenuated by 25 dB so that only the last bin in the water column was strongly affected by side-lobe reflection.)

As indicated by the 2.5-h data segment in Fig. 2, the acoustic signal consisted of isolated or irregularly grouped "targets" super-

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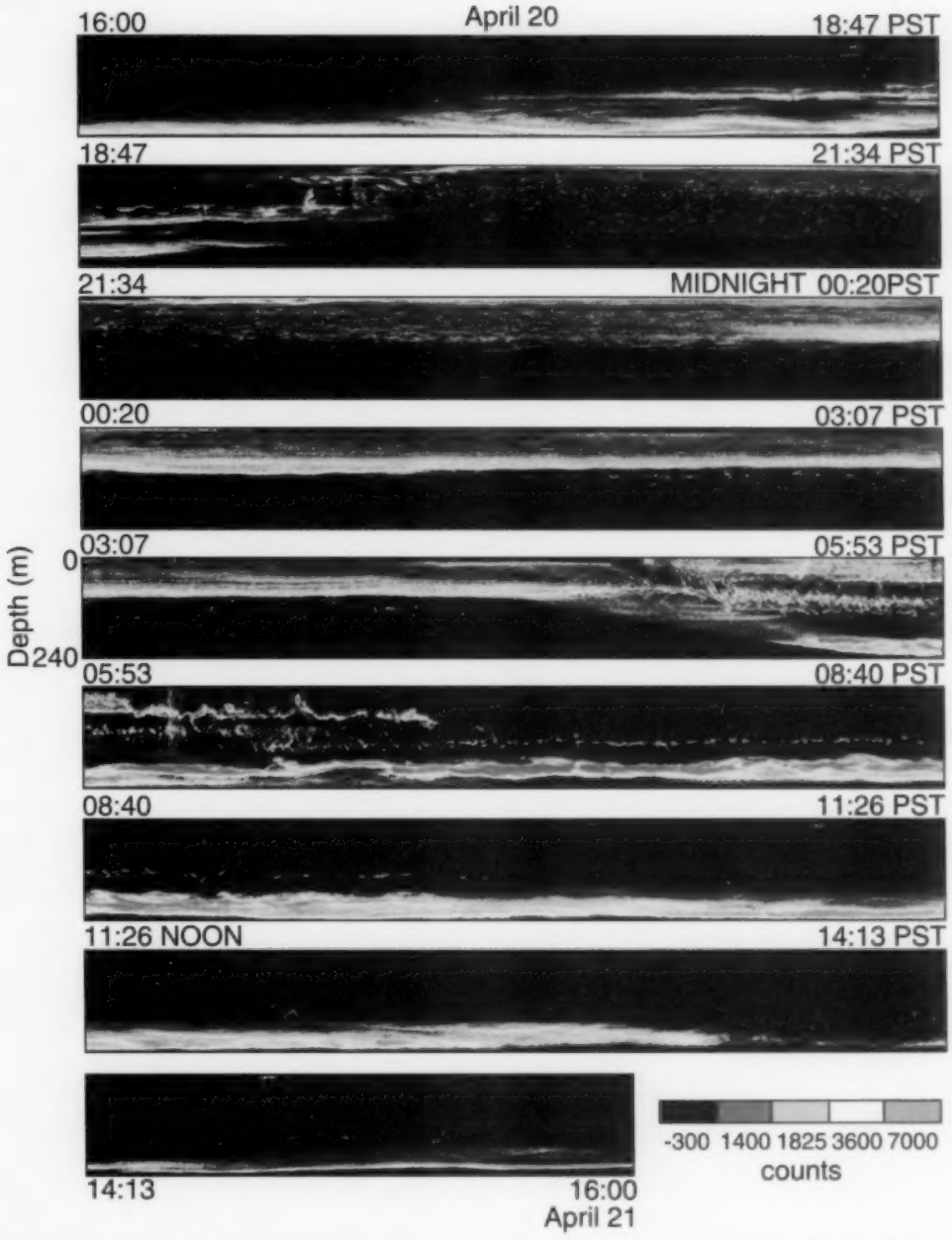
Fig. 4 (*concluded*).



imposed on strongly banded, temporally persistent, and acoustically "blurred" scattering layers. Each pixel in the acoustic image has a duration of 10 s and a height of 2 m. Because zooplankton in the study region would have been actively feeding during April (Mackas and Sefton 1982; Mackas et al. 1997), we have assumed that the scattering layers observed near the seafloor during daylight hours and near the ocean surface during darkness comprise vertically migrating zooplankton. The much more "jagged," high-intensity targets are assumed to be acoustic reflections from pelagic fish. Because the acoustic record is only 5 days long, we are presumably dealing with the same species and size distributions of zooplankton and fish throughout the observation period. Similarly, the water properties measured at the time of the sounder deployment were conditions that prevailed throughout the time series. Although longer time series are always preferred, they would also require additional information on possible changes in fish and zooplankton size distribution and species composition.

Target strength

For a target at distance r from the WASP transducer, the recorded backscatter signal, $N(r)$ (in counts), is related to the target

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strength, TS(r), of the acoustic scatterers through the sounder equation

$$(2)\quad 20\log[(5N(r)/255)/V_o] = C - 2[20\log(r) + \alpha(r)] + \mathrm{TS}(r)$$

where V_o is the transmit voltage, C is a constant for specified instrument parameters (e.g., transmit frequency, transducer gain, and transmit voltage response), and α (decibels per metre) is the attenuation coefficient of water at 165 kHz. The target strength is assumed to be a function of biomass. The instrument was programmed to send an acoustic "ping" with a pulse length of 500 μs every 5 s and to acquire a new reading every 50 μs for a total of 54 independent values per 2-m bin per 5-s ping based on a sound speed - c_s = 1465 m·s^{-1}. Each individual value is in the range 0–255 (8-bit "words"), and two successive 5-s samples were averaged to produce the 10-s backscatter intensity record stored in the instrument memory. Integration of the return signal over each 5-s interval yields an acoustic count with a maximum permissible value of 54 × 255 × 2 = 28 050 counts per bin. The WASP reserves a 15-bit location for all integrated binned data samples, with the 16th bit used to indicate if any of the samples collected within a bin during a given ping were "clipped" due to saturation of the sounder electronics.

Current meter records are available from the WASP mooring at approximately 230 m depth on the northern side of Barkley Canyon and from three additional sites within the canyon (Fig. 1). Shipboard conductivity – temperature – depth – optics – chlorophyll *a* (CTD-O-Ch-*a*)/rosette profiles of temperature, salinity, light attenuation, nutrients, dissolved oxygen, and other oceanic parameters were collected at the time of the WASP deployment. Tidal currents within the confines of the canyon are typically of the order of 1 cm·s^{-1}, with the tidal ellipses for both the diurnal and semidiurnal currents paralleling the cross-slope axis of the canyon. Because of a strong baroclinic tidal component, the amplitudes and phases of the semidiurnal currents undergo considerable variability with depth. In contrast, diurnal currents are dominated by a weakly baroclinic shelf wave component for which current amplitude and phase are nearly uniform with depth (Crawford and Thomson 1991). Down-canyon (toward approximately 240°T (T = true)), near-bottom residual (tide removed) flow persisted throughout the current meter time series at the WASP mooring site. For the 5-day period of the acoustic record, daily mean currents at this site ranged from 2 to 3 cm·s^{-1}. Several events with down-canyon currents in excess of 10 cm·s^{-1} were recorded later in the summer at this location. The persistent down-canyon flow at the WASP site combined with the persistent cross-canyon (eastward) flow at the mouth of the canyon and up-canyon flow along the axis of the canyon is consistent with a cyclonic eddy centered over the head of the canyon. Shallower currents over the shelf-break (<200 m depth) were mainly to the southeast in response to the northwesterly winds along the coast. Water property profiles collected in the vicinity of the WASP site in mid-April show a shallow (<50 m), highly stratified layer of relatively warm, low-salinity, chlorophyll- and nutrient-rich coastal water extending approximately 90 km offshore. Seaward of the boundary between the coastal and offshore oceanic regimes, the upper layer was weakly stratified to a depth of 60 m and overlay a marked pycnocline from 60 to 115 m depth (R.E. Thomson and S.E. Allen, unpublished data).

Calibration of the acoustic data

Because we are primarily interested in relative, rather than absolute, changes in backscatter intensity with time, t, we attempted to use in situ calibration procedures to estimate the count anomaly (or residual)

$$(3)\quad N'(r,t) = N(r,t) - \langle N(r)\rangle$$

relative to the background level $\langle N(r)\rangle$ (cf. Thomson et al. 1991). This procedure compensates for the r-dependent attenuation of the acoustic signal due to geometric spreading and acoustic absorption expressed by the term $20\log(r) + \alpha(r)$ in eq. 2. Removal of the range-dependent attenuation provides a quantitative estimate of the target strength for scatterers in the water column.

We first attempted to calibrate the acoustic signal using data recorded for each bin during descent of the instrument package to the seafloor. This type of in situ calibration has been used by Thomson et al. (1991) to calibrate the automatic gain control data from acoustic Doppler current meters. The method focuses on backscatter intensity measured by different bins but at the same depth in the water column. With sufficient overlapping data (i.e., most of the bins ensonify the same portion of the water column as the instrument falls to the seafloor), it is possible to obtain a statistically meaningful estimate of $\langle N(r)\rangle$. The difficulty faced in the present study is that the free-fall was too short and the vertical variation in zooplankton abundance too high for us to obtain reliable averages as a function of range for all but a few of the acoustic bins. We therefore simply averaged all data for each bin (a total of 49 000 records per bin) and used the record-averaged profile (dotted line in Fig. 3) as a measure of the range-dependent acoustic attenuation. This mean profile, which incorporates the effects of acoustic attenuation and the mean vertical distribution of diurnally variable biomass, has an approximate exponential decrease with distance from the transducer (solid line in Fig. 3). Subtraction of the mean profile from the original acoustic data using eq. 3 yields a record of pseudo-range-corrected backscatter intensity (Fig. 4).

Separation of fish and zooplankton

Visual inspection of the backscatter time series in Figs. 2 and 4 suggests that there were variations in zooplankton diel migration associated with day-to-day changes in the abundance and depth range of pelagic fish. In an attempt to quantify this effect, we developed a notch filter with adjustable threshold amplitude T (in counts), filter widths $n\Delta d$ (in depth) and $m\Delta t$ (in time), relative bin strength C (relative amplitudes of adjoining bins), and background noise level B (in counts) to separate fish pixels from background zooplankton pixels (Fig. 5). (Here, Δd = 2 m, Δt = 10 s, and n and m are positive integers.) Integration of the digital data series with respect to depth and time provides independent estimates of the fish and zooplankton biomass components. As with any filter, the selection of filter parameters is partially subjective. In our case, the threshold intensity and filter widths were obtained by trial and error, conforming to what we consider visually constitutes a fish or school of fish (acoustic targets with high localized intensity and (or) jagged edges). A sensitivity analysis was performed using a range of filter thresholds and relative bin strengths. The estimated numbers of fish varied by a factor of 2, but the basic pattern remained unchanged. Although the availability of multiple frequencies would have improved the selectivity of any fish filter, the marked difference between the fish and zooplankton signatures (high-intensity space–time localization versus low- to moderate-intensity and temporal banding, respectively) makes it possible to use a single frequency. Where there was overlap between fish and zooplankton (such as during the early morning descent of zooplankton through middepth where the fish appear to have been actively feeding), the fish are included within the zooplankton biomass counts. Our filter underestimates fish biomass and overestimates zooplankton biomass.

Results

Timing of zooplankton diel migration

During optically good atmospheric conditions, the sunlight incident at the ocean surface is determined mainly by

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Fig. 5. Notch filter algorithm with adjustable threshold amplitude T(counts), filter widths $n\Delta d$ (in depth) and $m\Delta t$ (in time) (n and m are positive integers), relative bin strength C, and background noise level B(counts) used to separate fish pixels from zooplankton pixels (Δd = 2 m, Δt = 10 s, n = 3, and m = 6). The flowchart on the right is a subroutine used at the two double-lined diamond sites in the main algorithm.

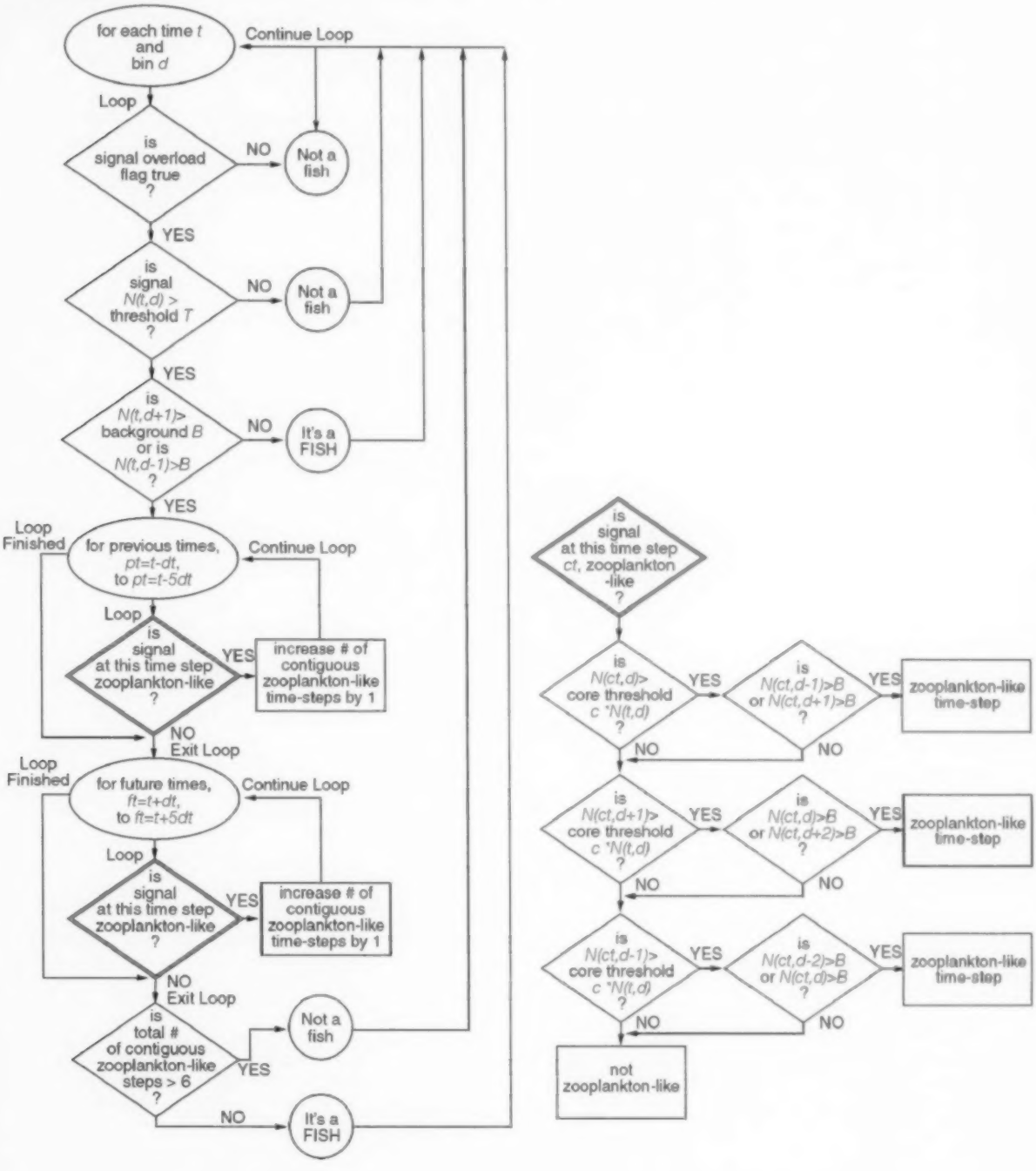

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Table 1. Stages of zooplankton migration at WASP mooring site BC04 (48°24.878′N, 125°56.837′W) from April 18 to 23, 1997.

Process	April 1997	Local time (PST) (+8 h)	UTC	Sunrise/ sunset	Nautical twilight	Fish abundance	Solar radiation ($W \cdot m^{-2}$)
Begin initial ascent	18–19	18:30	02:30			Low	
Begin rapid ascent		19:06	03:06	19:04		Low	100[a]
Mid rapid ascent		19:50	03:50				
End rapid ascent		20:04	04:04		20:17	Low	
Begin initial descent		01:05	09:05			Low	69% cloud
Begin rapid descent		04:40	12:40		04:00	Low	130[b]
End rapid descent		05:53	13:53				
End descent		08:53	16:53	05:13		Medium	
Begin initial ascent	19–20	17:00	01:00			Very low	
Begin rapid ascent		19:06	03:06	19:05		Very low	115[a]
Mid rapid ascent		19:27	03:27				
End rapid ascent		19:52	03:52		20:19	Very low	
Begin initial descent		23:42	07:42			Medium	87% cloud
Begin rapid descent		04:40	12:40		03:57	Very high	183[b]
End rapid descent		05:00	13:00				
End descent		08:17	16:17	05:11		Very high	
Begin initial ascent	20–21	17:58	01:58			Low	
Begin rapid ascent		19:27	03:27	19:06		Medium	157[a]
Mid rapid ascent		19:41	03:41				
End rapid ascent		20:00	04:00		20:20	Medium	
Begin initial descent		23:38	07:38			Medium	32% cloud
Begin rapid descent		04:46	12:46		03:55	Medium	270[b]
End rapid descent		05:40	13:40				
End descent		05:53	13:53	05:09		High	
Begin initial ascent	21–22	14:47	22:47			Very low	
Begin rapid ascent		19:21	03:21	19:08		Very low	85[a]
Mid rapid ascent		19:38	03:38				
End rapid ascent		20:06	04:06		20:22	Low	
Begin initial descent		01:40	09:40			High	40% cloud
Begin rapid descent		04:48	12:48		03:53	High	213[b]
End rapid descent		05:34	13:34				
End descent		07:29	15:29	05:07		High	
Begin initial ascent	22–23	10:46	18:46			Low	
Begin rapid ascent		19:08	03:08	19:09		Medium	155[a]
Mid rapid ascent		19:37	03:37				
End rapid ascent		20:07	04:07		20:24	High	
Begin initial descent		01:47	09:47			High	95% cloud
Begin rapid descent		04:48	12:48		03:51	High	170[b]
End rapid descent		05:29	13:29				
End descent		10:44	16:44	05:05		High	
Begin initial ascent		18:19	02:19			Medium	

Note: The instrument was located 16 m above bottom in 240 m of water. PST = UTC – 8 h. Fish abundance is a qualitative estimate of the number of fish based on the derived fish abundance time series. The last column gives 6-h incoming solar radiation for late afternoon (16:00–22:00, superscript *a*) and early morning (04:00–10:00, superscript *b*) plus percentage of cloud cover at night (22:00–04:00 PST).

the elevation of the sun. Sunrise and sunset are defined as the time when the center of the sun is geometrically 0.8333° below the horizon; nautical twilight — the time under clear atmospheric conditions when there is still sufficient light to distinguish objects on the ground — is defined as the time when the center of the sun is 12° below the horizon. Astronomical time tables available on the internet (www.usno.navy.mil/cgi-bin/aa_rstable) show that sunset times increased from 19:04 local time (Pacific standard time, PST) at the beginning of our acoustics record on April 18 to 19:09 PST at the end of our record on April 22. Sunrise times advanced from 05:13 PST on the morning of April 19 to 05:05 PST on April 23, leading to a net increase of 13 min in the hours of daylight through the course of the observations. Evening nautical twilight occurred slightly later each day of the acoustic record (from 20:17 to 20:24 PST), while morning nautical twilight occurred slightly earlier (from 04:00 to 03:51 PST).

We have chronicled the different stages of zooplankton migration in Tables 1 and 2 and summarized the results in a

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Fig. 6. Cartoon of diel zooplankton migration at the head of Barkley Canyon. The heavy solid line depicts the core of the zooplankton scattering layer, while the small isolated dots denote small fish that appear near the ocean surface during the night. Downward-migrating zooplankton descend more rapidly upon encountering the fish layers during the early morning hours.

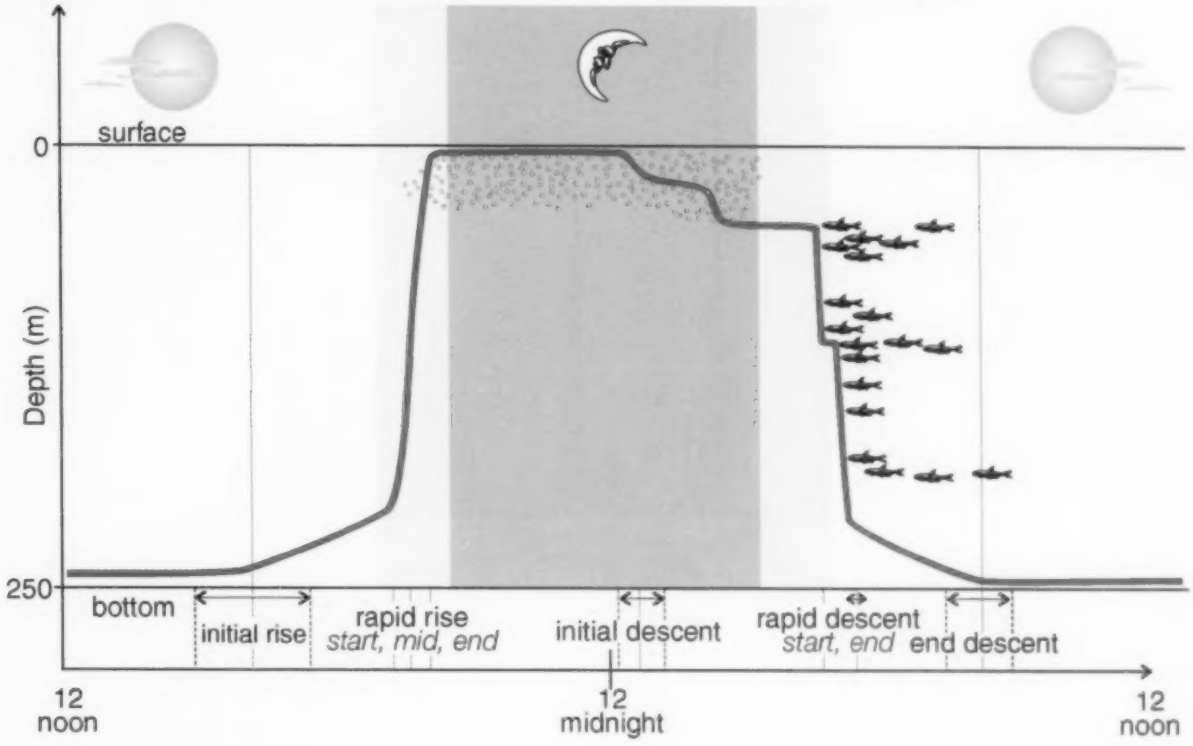

Table 2. Different stages of zooplankton migration and their time lag relative to the times of local sunrise/sunset and nautical twilight.

Process	Mean sunrise/ sunset time difference (min)	Mean nautical twilight time difference (min)
Begin initial ascent	–173.67±73.97	–248.00±74.08
Begin rapid ascent	7.20±4.22	–66.80±4.27
Mid rapid ascent	32.20±4.03	–41.80±4.26
End rapid ascent	55.40±2.32	–18.60±2.38
Begin initial descent	–260.60±27.67	–189.80±29.06
Begin rapid descent	–24.60±3.19	48.80±3.68
End rapid descent	22.60±8.73	96.00±9.60
End descent	186.20±48.30	260.00±53.97

Note: Values are expressed as the mean value (N = 4 or 5 samples) followed by the SE ($SD/\sqrt{N-1}$).

cartoon (Fig. 6). According to the acoustic time series, deep macrozooplankton aggregated in well-defined layers near the canyon floor during daylight hours (presumably to avoid predation), but sometime before sunset began to rise slowly from the seafloor, with initial ascent occurring as early as 10:46 PST (April 22) and as late as 18:30 PST (April 18). Although we cannot rule out effects of lateral advection, it appears that this initial liftoff is sometimes reversed. For example, during the afternoon of April 21, the core of the main scattering layer rose above the transducer depth only to settle back to the canyon floor several hours later, possibly because of the arrival of fish at middepth. In all cases, the slow-ascent stage terminated in a rapid and concerted ascent to the ocean surface beginning 7.2 ± 4.2 min after sunset or 66.8 ± 4.3 min before nautical twilight (expressed as the mean ± SE for N = 5 or 6 samples) (Table 2). During the rapid-ascent stage, the scattering layer was often fragmented or cloudlike, presumably because of horizontal patchiness in zooplankton abundance or because of a reduction in acoustic backscatter intensity caused by greater inclination of the euphausiids (Cochrane et al. 1994).

Based on the 5 days of data, the rapid-ascent stage ended 55.4 ± 2.3 min after sunset or 18.6 ± 2.4 min before nautical twilight. Excluding the ascent on April 19, when the maximum ascent rate was only 3.3 cm·s^{-1}, the maximum ascent rate was 6.3 ± 0.5 cm·s^{-1} and occurred roughly midway through the ascent stage, approximately 32.2 ± 4.0 min after sunset (41.8 ± 4.3 min before nautical twilight). We justify excluding the slower speed in our estimate of maximum ascent rate by noting that, on April 19, the bulk of the zooplankton were already midway through the water column before they began their rapid ascent and so did not have to swim as rapidly to arrive at the ocean surface at their "normal" time (roughly 18.6 min before nautical twilight). The rise of the zooplankton to shallower depths during the slow-ascent stage on April 19 appears to have been motivated by the presence of near-bottom fish. With the exception of April

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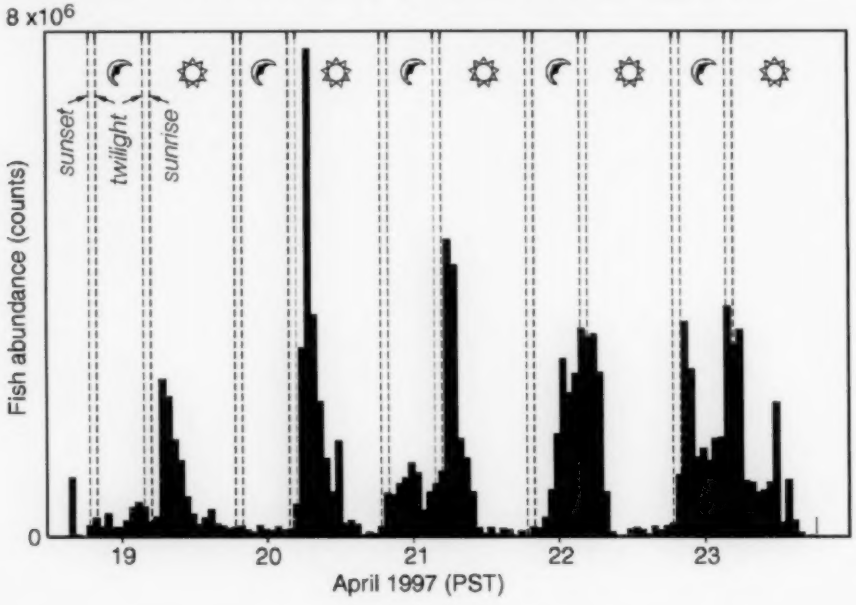
Fig. 7. Hourly fish abundance following application of the notch filter to the 5-day acoustic backscatter time series. Parameter settings for the filter were T = 2000 counts, $n\Delta d$ = 6 m (n = 3), $m\Delta t$ = 60 s (m = 6), B = 1000 counts, and C = 0.75.



19, fish were either absent or situated above the zooplankton layer prior to the rapid-ascent stage. April 19 was the only day that we observed significant numbers of fish below the depth of the zooplankton scattering layer during the ascent stage.

The animals remained in the upper portion of the water column during the night, often aggregating close to the ocean surface for much of the period of darkness. As indicated by Tables 1 and 2, zooplankton began their slow-descent phase during darkness 4.3 ± 0.5 h before sunrise and 3.2 ± 0.5 h before nautical twilight and their rapid-descent stage 24.6 ± 3.2 min before sunrise and 48.8 ± 3.7 min after nautical twilight. The sudden appearance of middepth scattering layers during the slow-descent stage suggests that zooplankton were moving (or being advected) laterally as well as vertically during this period. Unlike the rapid-ascent stage, the rapid-descent stage consisted of one or more steps, with the main body of zooplankton quickly descending just below the depth of any major fish layer. The average maximum descent rate of 6.4 ± 1.1 cm·s^{-1} was comparable with the average maximum ascent rate. The rapid-descent stage ended 22.6 ± 8.7 min after sunrise (96.0 ± 9.6 min after nautical twilight) and the zooplankton typically settled to the canyon floor within 2–4 h after sunrise.

Time series of fish abundance

We constructed an uncalibrated time series of integrated fish "biomass" (Fig. 7) by summing the measured fish counts over time and depth, limiting the integration to the upper and middle portions of the water column. To reduce the noise level, summation was over 1-h segments of the sequential 10-s data and spanned depth bins 35–115 (nominal depth 155 m to the surface). In general, the fish count was least during hours of darkness and greatest immediately after sunrise, with the maximum fish count (approximately 7.8 × 10^6) occurring just after sunrise on April 20. Superimposed on this daily cycle was a trend toward greater numbers of fish during hours of darkness, from a minimum of 1.3 × 10^6 fish during the night of April 19–20 to a maximum of 1.7 × 10^7 fish during the night of April 22–23. The time-integrated fish count for daylight hours varied from a minimum of 1.0 × 10^7 on April 19 to a maximum of 2.1 × 10^7 on April 20.

Two types of fish signal were observed in the digital acoustic "pixels": (*i*) small, but intense acoustic reflectors spanning 1–2 pixels and observed during the night when the zooplankton were near the ocean surface and (*ii*) large, intense acoustic reflectors spanning 5–10 pixels that appeared in the data series around dawn and remained in the upper portion of the water column above the zooplankton scattering layers for differing lengths of time through the day. On April 21, the large reflectors had disappeared from sounder view by 10:00 local time whereas on April 23, they remained until sunset. With the exception of the morning of April 20, when the small targets did not disappear entirely until the large targets appeared, there was no overlap between the two types of fish targets.

Discussion

Mackas et al. (1997) noted that "From spring through autumn, euphausiids and Pacific hake (*Merluccius productus*) form dense aggregations off the outer coast of British Columbia along regions of steeply sloping bathymetry such as the continental shelf break." Seasonal to interannual variability in zooplankton biomass and species composition for the

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Table 3. Six-hourly incoming solar radiation ($W \cdot m^{-2}$) and nighttime cloud (percentage of the sky covered) for the southwest coast of Vancouver Island (48.5°N, 126.0°W) in the vicinity of Barkley Canyon.

	April 1997				
Averaging period (local time, PST)	18	19	20	21	22
Late afternoon sun (16:00–22:00)	100	115	157	85	155
Morning sun (04:00–10:00)		130	183	270	213
Midday sun (10:00–16:00)		350	575	575	445
% night cloud cover (22:00–04:00)	69	87	32	40	95

west coast of Vancouver Island has been described by Mackas (1995). During April, euphausiids (mainly *Euphausia pacifica* and *Thysanoessa spinifera*) are the dominant diel-migrating species, while copepods are the dominant nonmigrating species (D. Mackas, Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, BC V8L 4B2, Canada, personal communication). We observed a similar species composition in net tows collected over Barkley Canyon in July 1997, several months after the end of the WASP time series (Vindeirinho 1998; R.E. Thomson and S.E. Allen, unpublished data). The "bulge" in our calibration curve centerd near 75 m depth (bin 75) over the depth range 25–100 m is the average backscatter intensity arising from fish targets as well as from migrating and nonmigrating zooplankton species. Because migrating zooplankton species quickly pass through this depth range during their diel migration and fish tend to disperse when feeding, the midwater bulge presumably arises from aggregations of nonmigrating zooplankton and from schooling of Pacific hake during the day (Kieser et al. 1996). In contrast, the enhanced calibration signal starting below about 165 m depth (bin 27) most likely arises from the persistent and structured aggregation of diel migrators during the day.

The 5-day, high-resolution acoustic anomaly data collected by the WASP in Barkley Canyon in late April 1997 suggest that diel migration of the dominant zooplankton species (euphausiids) consists of four primary stages: (*i*) the slow-ascent stage beginning with slow liftoff from the seafloor in the early evening, (*ii*) the 50-min rapid-ascent stage beginning 7.2 ± 4.2 min after sunset and ending 55.4 ± 2.3 min after sunset, (*iii*) the slow-descent stage beginning several hours before sunrise, and (*iv*) the steplike, 50-min rapid-descent stage beginning 24.6 ± 3.2 min before sunrise and ending 22.6 ± 8.7 min after sunrise. Maximum vertical migration rates occurred midway through migration periods and were roughly 6.4 $cm \cdot s^{-1}$ for both the rapid-ascent and rapid-descent phases. Our results differ slightly from those of Rippeth and Simpson (1998) for the Hebridean shelf. Using an acoustic Doppler current profiler, they found that the midpoint of most rapid ascents occurred at sunset, while the midpoint of most rapid descents occurred before sunrise.

The two main types of "fish" reflectors in the WASP records had markedly different acoustic intensity and behavior. Based on previous hydroacoustic studies for the southwest coast of Vancouver Island (Ware and McFarlane 1995; Kieser et al. 1996; Mackas et al. 1997), it is likely that the isolated, near-surface targets observed at night are individual Pacific hake or Pacific herring (*Clupea pallasi*), while the large targets observed in the early morning and during the day are schools of Pacific hake. Although we have no coincident catch data to verify these assumptions, others (e.g., Tanasichuk et al. 1991; Ware and McFarlane 1995) have determined that Pacific hake and Pacific herring are the dominant fish species off southwest Vancouver Island from spring through summer when they are feeding heavily on euphausiids. Except during the fall, when the euphausiid biomass has been depleted and the Pacific hake are seeking alternative food sources, there is minimal overlap between the two fish species in the region of Barkley Canyon (Ware and McFarlane 1995). Euphausiids and Pacific hake are strong vertical migrants and both have similar horizontal distributions with vertical separations of tens of metres during daylight hours (Mackas et al. 1997). The vertical "needle-shaped" acoustic distributions that we observed near the surface are indicative of Pacific herring aggregations (Kieser et al. 1996), while the zigzag structure of the large numbers of middepth fish targets during daylight hours is indicative of Pacific hake (Kieser et al. 1996; D. Ware, Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada, personal communication).

Although diel zooplankton migration appears to be mainly governed by ambient light conditions, day-to-day variations in ascent and descent times are clearly linked to the numbers and depth range of pelagic fish in the water column at the time of migration. This is especially true of the descent stage, when the predators (mainly Pacific hake) were most prevalent. Upon encountering large aggregations of fish, the zooplankton quickly descended until they were immediately below the depth of the middepth predators. If there were few fish in the water column during the descent stage, the zooplankton only slowly moved to their daytime resting depth near the canyon floor. The one day with "anomalous" ascent behavior (the evening of April 19) was also linked to the presence of fish. On this day, the zooplankton had risen close to middepth before beginning their rapid ascent to the surface, suggesting that they were being forced upward by significant numbers of underlying fish.

To determine if day-to-day variations in diel migration were related to weather-related changes in ambient light conditions rather than fish abundance, we compared the timing of the ascent and descent stages of the zooplankton migration with the intensity of incoming solar radiation and nighttime cloud cover (6-h average incoming radiation data for location 48.5°N, 126.0°W were found on website www.cdc.NOAA.gov/cdc/data.nmc.reanalysis.html; U.S. National Meteorological Center). Based on the radiation data, the observational period was mainly cloudy with occasional clearing (Table 3). The brightest days were April 20 and 21 and the cloudiest day was April 19; the moon was full on April 22 and the clearest nights were April 20–21 and 21–22 (Table 3). The only correlation that we could find between migration and solar radiation was between the end of the descent stage and the intensity of the morning sunlight, with stronger sunlight possibly linked to an earlier end to the morning migration. If there was indeed a link between early morning solar radiation and the final descent stage, it more likely was associated with the fish by enhancement of their

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ability to find prey. This, in turn, presumably influenced the downward migration of the zooplankton.

Because zooplankton are closer to the surface during the night, moonlight might be expected to affect the timing of the descent stage. Such a linkage has been reported by Tarling et al. (1999) who found that the initial descent of the euphausiid *Meganyctiphanes norvegica* takes place at moonrise. In our case, there was no correlation between diel migration and nighttime cloud cover, and moonrise occurred before the zooplankton had reached the ocean surface.

Generalized results of this study are as follows. If there are no fish aggregations in the lower portion of the water column, zooplankton begin their diel cycle by rising slowly from the seafloor hours before sunset. Roughly 5 min after sunset, the animals undertake a rapid ascent from below middepth (approximately 100–150 m), arriving near the ocean surface about 55 min after sunset, or about 19 min before nautical twilight. High concentrations of relatively small fish often appear in the upper portion of the water column at the end of the rapid-ascent stage. The zooplankton remain in the upper ocean for much of the night before beginning a slow descent several hours before sunrise. Whereas the slow-ascent stage was only partially affected by pelagic fish (on the evening of April 19, significant numbers of deep fish caused the zooplankton to ascend all the way to middepth before undertaking a rapid ascent), the descent stage appears to be strongly linked to the abundance of fish in the water column. The rapid-descent stage begins 25 min before sunrise and continues in steps until about 25 min after sunrise. Major steps in zooplankton depth coincide with an encounter with large aggregations of fish. Maximum vertical migration rates of around 6.4 $cm \cdot s^{-1}$ occur around middepth, midway through the rapid-ascent and rapid-descent phases. Zooplankton tend to be several tens of metres deeper than the major fish aggregations except during brief periods of encounter during the zooplankton descent stage. During daylight hours, fish and zooplankton remain well separated vertically (cf. Mackas et al. 1997).

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PERSPECTIVES

Critical analysis of water quality monitoring in the Russian Federation and former Soviet Union

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Abstract: Water quality in Russia has both domestic and international consequences. Domestically, it allows for appropriate management of aquatic systems; internationally, surface flow from present Russian and former Soviet Union territory into international waters (e.g., Arctic Ocean and Aral, Black, and Caspian seas) has important implications for global contamination levels and for developing future management plans. Although during the Soviet era the Russian water quality monitoring network was one of the most extensive in the world, numerous anomalies identified in Russian data by domestic and foreign scientists have been referred to the authors for comment. A holistic assessment of the purpose and current status of the Russian water quality monitoring program is essential because of the difficulty that "outsiders" have in obtaining unbiased information about the program and because this is the principal historical database on water quality that is available for the former U.S.S.R. and Russian Federation. Apart from chronic underfunding, the main problems that need to be addressed are poor functioning of the system, including network design, choice of parameters, sample collection, analytical conditions and data quality, data handling, data products, and issues of access, and the larger question of institutionalized flexibility required to meet local data needs.

Résumé : La qualité de l'eau en Russie a des conséquences à la fois nationales et internationales. À l'échelle nationale, elle permet de gérer adéquatement les systèmes aquatiques; à l'échelle internationale, l'écoulement superficiel provenant du territoire de l'actuelle Russie et de l'ancienne Union soviétique dans les eaux internationales (océan Arctique, mer d'Aral, mer Noire et Caspienne) a d'importantes incidences pour le niveau de pollution planétaire et pour l'élaboration des futurs plans de gestion. Si pendant la période soviétique, le réseau de surveillance de la qualité de l'eau en Russie était l'un des plus étendus du monde, de nombreuses anomalies repérées dans les données russes par des chercheurs russes et étrangers ont été signalées aux auteurs. Une évaluation globale de l'objectif et de l'état actuel du programme russe de surveillance de la qualité de l'eau est essentielle, étant donné la difficulté pour les gens de l'extérieur d'obtenir une information impartiale sur le programme, et du fait qu'il s'agit là de la principale base de données historiques sur la qualité de l'eau qui existe pour l'URSS et la Fédération de Russie. Mis à part le sous-financement chronique, les principaux problèmes à résoudre sont le mauvais fonctionnement du système, ce qui inclut la conception du réseau, le choix des paramètres, la collecte d'échantillons, les conditions d'analyse et la qualité des données, la manipulation des données, les produits qui en sont tirés et les problèmes d'accès, ainsi que la plus large question de la souplesse institutionnelle nécessaire pour répondre aux besoins locaux en données.

[Traduit par la Rédaction]

Introduction

The Russian water quality monitoring system during the Soviet era was one of the most extensive in the world. Internationally, surface flow from present Russian and former Soviet Union territory into international water bodies, such as the Arctic Ocean and Aral, Black, and Caspian seas, has important implications for global levels of contamination and for developing future management plans. Prior to the 1990s, most scientists (Russian or otherwise) were unable to access,

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analyse, or publish official water quality data of the former Soviet Union. While such restrictions no longer exist, much of the data remain inaccessible (see below). With a growing international interest in Russian water quality data (e.g., Kimstach et al. 1998; Zhulidov et al. 1998; Holmes et al. 2000) and Russia's participation in global water quality and assessment programs such as GEMS/Water (Global Environment Monitoring System) (www.cciw.ca/gems) and the Arctic Monitoring and Assessment Program (AMAP) (www.grida.no/amap/amap.htm), it is timely to provide a holistic assessment of the purpose, current status, and reliability of the Russian water quality monitoring program. The assessment focuses only on surface water quality, as this has been the mandate of the national program over the long life of the program. Facts and opinions of the authors are based on an extensive involvement with (and for one of the authors, inside) this program over many years and quantitative analysis of certain aspects of the monitoring program such as data quality.

An important feature of the current federal surface water monitoring system of the Russian Federation is that it evolved from the system of environmental control of the former U.S.S.R. Therefore, a historical context is essential to the understanding of current problems inherent in monitoring programs and practices in Russia.

Historical outline

The State Service of Observation and Control of Environmental Pollution (OGSNK), which from 1992 became the State Service of Observation of Environmental Pollution (GSN), was formed in 1973 by the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers of the U.S.S.R. and was mandated to measure the contamination levels of the atmosphere, soils, surface waters, and seas. OGSNK development was under the supervision of the Hydrometeorological Service (Glavgidrometsluzhba) of the Council of Ministers of the U.S.S.R., which de facto became the principal agency for environmental control in the U.S.S.R. The organizational basis for OGSNK was envisaged at the time as the monitoring, operational, and scientific components of organizations under the control of the Council of Ministers from the Ministry of Public Health, Ministry of Land Reclamation and Water Economy, Ministry of Agriculture, and several other agencies (Izrael et al. 1978). Initially, OGSNK comprised 3529 Glavgidrometsluzhba sites and 148 sites that formerly belonged to other departments (Tsirkunov 1998*a*).

Institutionally, the program has undergone many changes over the years. In 1978, the Hydrometeorological Service was reorganized into the State Committee of the U.S.S.R. for Hydrometeorology and Natural Environment Control (Goskomhydromet), which in turn was renamed the State Committee of the U.S.S.R. for Hydrometeorology (the name Goskomhydromet was retained) and then the Hydrometeorology Committee at the Cabinet of the U.S.S.R. (Goshydromet). From November 10, 1991, until September 1992, Goshydromet was called the Committee for Hydrometeorology and Environmental Monitoring (Roskomhydromet). For a few months, Roskomhydromet was part of the Ministry of Ecology and Natural Resources of the Russian Federation. From September 30, 1992, Roskomhydromet was reorganized into the Federal Russian Service of Hydrometeorology and Environmental Monitoring (Roshydromet). In April 1998, Roshydromet was abolished and its functions given to the Federal Environmental Protection Committee of the Russian Federation, which was then disbanded on May 19, 2000, and its functions assigned to the Ministry of Natural Resources. Roshydromet was restored on September 30, 1998, with some reporting requirement to the Ministry of Natural Resources but with considerable independence insofar as the Head of Roshydromet reports directly to the government. This can and does make for an uneasy situation between the two organizations. In all discussions below, we refer to the national monitoring program as Roshydromet.

In 1974, the Hydrobiological Service of Observation and Control of Surface Waters was formed as part of OGSNK. The main goal of the Hydrobiological Service was, and remains, to observe the condition of water bodies in terms of hydrobiological (and later toxicological) properties as well as to detect changes in aquatic communities under anthropogenic influence (Izrael 1984).

The key aims of OGSNK, and of the present hydrometeorological service, Roshydromet, are "acquisition of objective information to enable an assessment of temporal and spatial environmental pollution levels within the USSR, for emergency information on drastic changes in pollution levels in order to take protective measures, and share all acquired information with stakeholders. Other functions of the service are making forecasts and informing concerned organisations and the general population of the possibility of a sharp increase in the pollution of air and surface and marine waters" (Izrael et al. 1978).

The observational system was based on a large, fixed-site network of stations across the country. Data was "aimed at evaluating the effectiveness of the activities targeted for environmental protection and served as a basis for long-term forecasts of the environmental situation in the country" (Izrael et al. 1978). This remains the focus of this program under 1998 Federal Law "On hydrometeorological services" (No. 113-FZ). The focus was on surface ambient conditions and not on effluents. Surveys were included in hydrometeorological operations, especially in the 1970s and early 1980s for specific purposes, but were not used as part of the operational monitoring program. All survey activities ceased in the 1990s.

The legal basis in Russia for water quality monitoring is relevant to this discussion. In the past, the only legal entity for long-term monitoring and assessment was Roshydromet. In 1997 monitoring responsibility was officially allocated both to Roshydromet and to the Ministry of Natural Resources. The exact jurisdictional boundaries between the two organizations are often unclear. Federal Law No. 113-FZ of the Russian Federation enabled monitoring by other organizations (e.g., institutes and individuals) that are licensed for such activities. However, this law focuses almost exclusively on long-term monitoring of the type historically associated with Roshydromet, and we could find no official use of evaluation data obtained by other organizations. For effluents, 1991 Federal Law "On environmental protection" granted

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monitoring rights to individuals and nongovernmental organizations and gave them access to environmental protection information.

In retrospect, it is easy to understand how monitoring was developed to facilitate the evaluation of status, trends, and aquatic response to remedial interventions on a large scale but not to the provision of data that are needed for day-to-day operational management of water quality at the local level. As we show below, it is this gap and the lack of an appropriate institutional response that has been the Achilles heel of water quality management both formerly and presently in the Russian territory. Nevertheless, it can be said that this major program of environmental monitoring played an important role in environmental protection in the former U.S.S.R.

Operational principles of Roshydromet

The operating principles of Roshydromet are inherited from its predecessor institutions and are reflected in Federal Law No. 113-FZ. Therefore, the following observations reflect the historical as well as the current situation.

According to Izrael et al. (1978), the total number of OGSNK sites was about 4000 in the mid-1970s and covered approximately 1200 water bodies. Nevertheless, in a territory as large as the Soviet Union, the density of sites was always quite low. At the beginning of the 1990s, one OGSNK observation site covered an area of 9100 km^2 (on average). For the central European part of Russia, this coverage was one site per 1300 km^2, and one site in Yakutia and one site in the Russian Far East covered an area of 330 000 and 167 000 km^2, respectively. In comparison, the Finnish EUROWATERNET has 437 observation sites with coverage of one site per 770 km^2 (Niemi et al. 2000).

At present, some GSN observation sites (about 10%) (Anonymous 1997*b*) have had all activities "temporarily suspended." However, the number of GSN observation sites has not decreased (although the number differs depending on the source), as they remained the same since 1991, ranging from 1892 in 1993 to 1931 in 1996. According to Roshydromet (www.mecom.ru/roshydro/pub/rus/index.htm), in 1996, the number of sites was 1795 whereas in 1998, it was 1928 ("State of the environment in the Russian Federation in 1998 (www.gov.ru/ministry/isp-vlast47.html)). In the official report of the Roshydromet chief, "Summary of Roshydromet activities in 1998" (www.mecom.ru/roshydro/pub/rus/index.htm), it is stated that activities are not temporarily suspended but that the observation sites are closed down. In this summary, the number of monitoring sites in 1997 was 1714 and 1998 was 1699. As a result of this temporary suspension (or closing down) and low financing of monitoring sites, more than 25% of the monitoring network was financed by local government budgets.

Principle of integrated observations

Water quality and quantity sampling together with hydrological and biological (10% of sites) measurements were (and are) always done at fixed ("stationary") sites. The requirement for fixed sites is laid out in GOST (Federal Standard) (1982) and Anonymous (1984). Integrated sampling on site became known as "the principle of integrated observations" (Izrael et al. 1978). However, hydrological characteristics for hydrochemical observation sites were sometimes given without a hydrological estimation error (O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication). Frequently, hydrological parameters for these sites were "estimated" using data from the nearest hydrological site, which could be at a considerable distance (O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication).

Principle of systematic observations

This principle was originally defined by OGSNK to mean the performance of observations at "strictly established sites and in a determined time" as "only systematic observations allow the identification of spatial and temporal patterns of pollutant distribution" (Izrael et al. 1978). Permanent sites could not be closed without reasons. In the past decade, however, Roshydromet has not been able to maintain this principle, mainly due to technical and financial reasons. Fieldwork (assessment programs) once formed a small component of the national program but was eliminated from program activities in the 1990s.

The use of fixed-site networks has been challenged since the 1980s as being inefficient, and most modern monitoring programs in developed countries now use some combination of fixed sites and surveys. However, in Russia, the Roshydromet program has retained a primary focus on its fixed network.

Principle of standardized measurement methods

This principle was formulated as follows: "The importance of unified observation methods in the activities of the state service is obvious. To make data on pollution levels for various elements of the environment comparable...all work methods are unified and obligatory for the federal service" (Izrael et al. 1978). Nationwide, all samples were analysed in accordance with Roshydromet official methods in regional Roshydromet laboratories. In 1990, 33 829 water samples were collected and 1 098 317 analyses were made of 122 parameters from 4556 observation sites and 4995 vertical sections situated on 2258 water bodies, including 1970 rivers, 145 lakes, and 143 reservoirs (Tsirkunov 1998*a*). During 1991–1996, the total number of water samples analysed ranged from 32 919 in 1991 to 24 537 in 1996. The total number of measured parameters varied from 122 to 130, but the total number of analyses steadily decreased over this period from 1 047 599 in 1991 to 621 103 in 1996.

Analytical data are sent to regional computation centres to be copied to magnetic tapes. Copies of the tapes are sent to the Hydrochemical Institute, which holds the national database on surface water quality in the Russian Federation (and the U.S.S.R.). Regional Roshydromet departments prepare, publish, and disseminate among stakeholders informational documents (i.e., "express information," "general information," "specialized information" in accordance with Federal Law No. 113-FZ) on surface water quality for the territories concerned. Reviews of surface water quality covering physical and chemical parameters (see below) for all of Russia are prepared by the Hydrochemical Institute, while hydrobiological data are the responsibility of the Institute of Global Climate and Ecology. Besides routine information,

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Table 1. Characteristics of observations and analyses performed for various Roshydromet monitoring categories (Tsirkunov 1998*a*).

	Monitoring program for different site categories			
Observation frequency	Category I	Category II	Category III	Category IV
Daily	Short program 1	Visual observations		
Every 10 days	Short program 2	Short program 1		
Every month	Short program 3	Short program 3	Short program 3	
During major hydrological events	Obligatory program	Obligatory program	Obligatory program	Obligatory program

Note: Short program 1: water discharge (for streams) or water level (for lakes and reservoirs), visual observations, temperature, specific conductivity, dissolved oxygen; short program 2: short program 1 plus pH, suspended matter, chemical oxygen demand, biochemical oxygen demand, concentration of two or three pollutants typical for the particular site; short program 3: short program 2 plus stream velocity (for reference discharge measurements), concentration of all pollutants for the particular site; obligatory program: short program 2 plus stream velocity (for reference discharge measurements), colour, transparency, odour, scale, redox potential, dissolved gases (oxygen and carbon dioxide), major ions (Cl^-, SO_4^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}, Na^+, K^+), total dissolved solids, nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^-), total iron, silicon, widely distributed pollutants (petroleum products, volatile phenols, heavy metals).

Roshydromet provides operational express information on surface water quality when water bodies are highly contaminated.

Raw data coming from regional Roshydromet departments are put into the national database without critical assessment. Infrequently, the Hydrochemical Institute screened out some data (O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication). After collation, the data are statistically processed, and annual reports on the condition of water bodies are published (e.g., Anonymous 1997*a*, 1997*b*, 1998). The number of copies was (and remains) limited to 250, mainly for reasons of cost. This restricts access to the information. Access to data other than what are contained in the annual reports may be purchased from Roshydromet.

Principle of organizing observations regarding contamination of natural environments

This principle was formulated as follows: "Observation sites are organised in capitals of the Union and autonomous republics, regional centres, large industrial cities, on water bodies and in agricultural regions affected by economic activities, and in areas of minimal pollution (background observations)" (Izrael et al. 1978).

Accordingly, monitoring sites are situated to reflect cities and large industrial towns, near waste water discharges of individual plants, mines, electric power stations, and other industrial enterprises, spawning grounds of valuable game species, predam river stretches, crossings of federal borders and rivers, representative large and small rivers, inflow areas of polluted tributaries of large lakes and rivers, and pristine areas such as biosphere reserves (Anonymous 1984, 1992).

At sites considered to be prone to pollution, sampling was more frequent than in smaller communities or in biosphere reserves. Furthermore, Roshydromet focused its monitoring on "large" rivers (especially in the latest years), so that the database mainly encompassed large pollution sources (www.mecom.ru/roshydro/pub/rus/index.htm). According to GOST (Federal Standard) (1977), large rivers had a catchment area of >50 000 km^2 and a discharge of >100 $m^3 \cdot s^{-1}$ and "medium" rivers had a catchment area of 2000–50 000 km^2 and water discharge of 5–100 $m^3 \cdot s^{-1}$; therefore, "small" rivers were those with values less than these.

The national network is highly biased towards polluted reaches of rivers and lakes. Therefore, the ability to extrapolate water quality conditions over the entire nation is limited. Surface water quality assessment is mainly focused on exceedances to the official maximum allowable concentration or when data indicate "high" or "extreme" levels of contamination. Annual books provide an interpretation of reasons for unfavourable surface water quality conditions and their changes and list the most polluted water bodies. At a higher level of aggregation, water quality and other environmental information is acquired from all agencies and synthesized by a Federal Environmental Protection Committee. Since May 2000, this function now resides with the Ministry of Natural Resources.

Summary of operating principles

In general, the simple principle of "measure everything everywhere" was not capable of successful implementation. Selection of sites produced a systematic bias in the database, and water quality data problems (discussed below) have compromised the database to the extent that, in general, it is not possible to make an accurate assessment of status and trends of the nation's surface water. An exception appears to be the long-term analysis of major ion concentrations in the water of certain rivers in the former U.S.S.R. (Tsirkunov et al. 1992; Tsirkunov 1998*b*; V.V. Tsirkunov, World Bank, Moscow Office, Russia, personal communication).

Data and data quality

Sampling and analysis included 35–40 physical and chemical parameters and were performed at all sites, as a rule, during the main hydrological phases. Generally, this involved four samples per year for lakes and reservoirs and six or seven samples per year for rivers. Sites were classified into four categories that prescribed sampling frequency and the parameters to be measured (Table 1). In 1990, the network comprised 22 category I stations, 62 category II stations, 1347 category III stations, and 1951 category IV stations (Tsirkunov 1998*a*). By 1993, there were 12 category I stations, 40 category II stations, 709 category III stations, and 1131 category IV stations (Tsirkunov 1994). The sampling frequency was presumed to be adequate for identifying trends but is known to be compromised often by local logistical and other factors.

Over the years, there has been a deterioration in the quality of collected raw data, partly because of inadequately trained personnel, poor quality of analytical equipment and reagents, imperfect quality assurance and quality control

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Table 2. Results of a comprehensive analysis of the historical database for the Lower Don River region carried out by the Centre for Preparation and Implementation of International Projects on Technical Assistance (CPPI), Rostov-on-Don, Russia (Boeva et al. 1999, 2000; A.V. Zhulidov, unpublished data).

There is no metadata file that indicates which analytical methods are associated with which data entries when different Roshydromet laboratories for the same parameter use different methods
Lengthy shipping and storage times are a source of error
No systematic validation to eliminate manual transcription errors in the originating laboratory or at the Hydrochemical Institute when compiling the national database
Very poor physical condition of many Roshydromet laboratories, especially since 1990
Very old equipment, especially for more advanced analyses such as gas chromatography, atomic adsorption spectrophotometry, etc. For some parameters such as organochlorine pesticides, the detection limit is far too high to be meaningful
Uncertain quality of chemicals and reagents
Many analytical methods certified for use in Russia are obsolete
Organochlorine analyses are performed on water samples when, in fact, organochlorine compounds are mainly associated with the sediment phase in water
Percentage of data that is considered reliable: nitrogen, 50%; phosphorus, 70%; BOD_5, 75%; organochlorine pesticides, 30%
The entire dissolved metals database is in error (by up to 2600 times for cadmium)
NO_2 and soluble forms of phosphorus, which are very unstable, were analysed in the laboratory and not in the field, so the data are inherently unreliable
Organochlorine pesticides contain large errors, leading to false conclusions such as "no pesticides in water" or, alternatively, levels that are much higher than actual

procedures in many Roshydromet laboratories, and, in recent years, a loss of qualified personnel due to economic hardship. Results of "external" control of quality assurance and quality control procedures carried out by the Hydrochemical Institute in OGSNK/GSN regional departments were sometimes unsatisfactory, but this was not reflected in the OGSNK/GSN database (O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication; V.F. Gekov, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication). Nor was information on the new methods that were introduced by Hydrochemical Institute specialists into regional OGSNK/GSN departments contained in the OGSNK/GSN database. New methodologies often caused considerable changes in the quality of chemical information (L.V. Boeva and Y.Y. Vinnikov, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication).

Lack of data reliability has been commented on in several critical analyses of the Roshydromet database (e.g., Bodo 1998; Zhulidov et al. 1998; Holmes et al. 2000). In a comprehensive analysis of the historical database for the Lower Don River region carried out by CPPI for a World Bank program, a number of general observations were made about issues ranging from the lack of metadata files on the methods used to the quality of specific data (Table 2). These are applicable to the entire Roshydromet program.

Indirect evidence for error includes comparative analysis with other river systems. Flux of ammonium ions (estimated for nitrogen) by the Rivers Ob (near the outflow at Salekhard) and Yenisey (near the outflow at Dudinka) exceeded those for the Amazon River by two and 1.6 times, respectively, even though the discharge of the Amazon is 16 times higher than that of the Ob and 12.6 times higher than that of the Yenisey (Holmes et al. 2000).

Errors affecting nutrients, phenols, oil products, surface active materials, heavy metals, and some other substances arise not only at the stage of analytical detection, but also during sampling, transportation, storage, and sample preparation. Collection and transportation of water samples to hydrochemical laboratories for analysis (in 75% of cases, sampling is done by the Roshydromet Hydrological Service) are critical links in the federal monitoring system. According to the 1993 official data, for 30–35% of the sites, the time of sample transportation to hydrochemical laboratories exceeded 10 days (Tsirkunov 1994). Our own observations and expert assessments of our colleagues suggest that this percentage is considerably higher (L.V. Boeva and Y.Y. Vinnikov, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication).

The situation for assessing the state of the Russian aquatic environment is particularly alarming, as the responsibility for the reliability of water chemistry data in the Russian Federation is officially assigned to Roshydromet (Government Prescript No. 555, May 20, 1999). Of 230 analytical laboratories in 1998, only 87 were certified. Some 10 000 analytical instruments are obsolete and maintenance is not carried out regularly. During the last 5 years, there has been no centralized procurement. Any instruments that were purchased used local budgets and environmental funds met only 1% of the demand (official report of the Roshydromet chief, "Summary of Roshydromet activities in 1998" (www.mecom.ru/roshydro/pub/rus/index.htm)).

The issue of reliability of OGSNK/GSN measurements arises not only for water chemistry data, but also for suspended matter. For example, official data assessment of 16 rivers at sites located nearest to their outflows (Kolyma, Indigirka, Bolshoy Anyuy, Anadyr, Alazeya, Lena, Mezen, Severnaya Dvina, Ob, Yenisey, Olenek, Onega, Pechora, Pur, Taz, and Yana) from 1980 to 1998 had data sets containing concentrations below the limit of quantification. This indicates that the officially prescribed methodology (Anonymous 1995) had not been followed and data quality control had been ineffective.

The data transmission procedure from Roshydromet regional departments to the Hydrochemical Institute, where the data acquired the status of official "OGSNK/GSN information," is particularly susceptible to error. Analytical re-

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sults were transcribed by hand into a ledger. These entries were then transferred to magnetic tapes, usually by technicians untrained in water chemistry. Once on tape, the data were to be verified for transcription errors by specialists from regional Roshydromet offices. This was not automated and our information suggests that the performance of this verification step was variable, depending on the particular regional office. Once the tapes entered the Hydrochemical Institute, the information was almost never checked or corrected. The procedure remained unchanged until the end of 1997. In 1998, Roshydromet regional departments started using personal computers in their work, which probably decreased the number of misprints in the GSN information (O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication; V.F. Gekov, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication), although transcription error analysis remains mainly without automation.

An example of data quality is illustrative. In 1995–1997, Hydrochemical Institute specialists were of the opinion that 20% of the data were questionable or grossly in error. Nevertheless, no record of these findings was entered into the official OGSNK/GSN database (V.F. Gekov, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication; O.A. Klimenko, Hydrochemical Institute, Novocherkassk, Russia, personal communication; A.A. Zenin, Hydrochemical Institute, Rostov-on-Don, Russia, personal communication).

Data availability and dissemination

Russia has a long history of limiting access to raw data for any users (including specialists internal to Roshydromet). This has had the effect of retaining a monopoly on monitoring information. Federal Law No. 113-FZ on monitoring states that "information on the quality of the environment, its contamination and informational products is open and generally accessible outside the information classified by the legislation of the Russian Federation as that of limited access." Provision of information services by Roshydromet is regulated by Russian Federation Government Prescript No. 1425, November 15, 1997, in which Roshydromet is permitted to sell information to nonfederal users (Russian Federation Government Prescript No. 555, May 20, 1999). This policy has also been applied to federal users. For Russia, this policy further devalues the data by making them generally unavailable.

Disconnect between monitoring data and water quality management

In many countries, national water quality programs tend to be disconnected with users of water quality information (Ongley and Ordoñez 1997). In Russia, one of the largest weaknesses of the federal water quality monitoring program is the gap between the function of monitoring and the process of making management decisions. Historically, water quality data collected by Roshydromet and its predecessor organizations were not linked to the need for data to manage water quality at the local or regional level. Indeed, the legal framework and current institutional structure specifically disconnect the functions of monitoring (Roshydromet) by the national program from the functions of water quality management (Ministry of Natural Resources). It is curious that Federal Law No. 113-FZ specifies "Federal water monitoring is aimed at information support of management and control in the field of water use and protection." However, the technical and administrative content of the law virtually precludes this goal.

For public health purposes, the federal monitoring program has never been linked to evaluation of compliance with Ministry of Health objectives for public health. Yet ironically, Federal Law No. 113-FZ clearly states that the activities of the Hydrometeorological Service should comply with the aim of public health protection. Hydrobiological and toxicological methods of surface water quality assessment have no legal basis in the Russian Federation and are only a recommendation within Roshydromet (Anonymous 1994).

The idea formulated by Ward (1989) that water quality monitoring as a means of assessing physical, chemical, and biological water characteristics be closely related to informational requirements of water quality management (Andriaanse 1997) was not encompassed in the federal monitoring system in the U.S.S.R. or Russian Federation. Recommendations of the Economic Commission for Europe (1996) on the "monitoring cycle" and its intimate linkage to water quality management also had no impact in Russia.

It is interesting to note that Russian Federation Resolution No. 307 "On performing federal water monitoring" (March 14, 1997) states "Federal water monitoring is aimed at information support of management and control in the field of water use and protection." Our experience shows that it did not cover the gap between monitoring and water resources management.

The national database has been used for management purposes only in the sense that (*i*) background levels have been used to determine maximum allowable concentration values on the questionable assumption that any values above these imply risk to the aquatic system and (*ii*) to indicate (in theory) at regional and national scales if there is deterioration from anthropogenic impacts or improvement due to conservation measures. As we observe in this paper, the database is not sufficiently reliable, continuous, or inclusive to meet either of these objectives. Similarly, as Ongley (1998) has noted, such databases are descriptive and not prescriptive and cannot be used for making investment decisions for remediation. Management of waste water discharges uses a different set of monitoring principles that have their own problems but are not discussed here.

In spite of the fact that it is still impossible to calculate the cost of monitoring work in the Russian Federation, it is obvious that the cost of acquiring data on surface water quality is increasing, and the value of federal information for water quality management, for example, is depreciating. In both the U.S.S.R. and Russian Federation, the syndrome of "much data but little information" (Ward et al. 1986) is very evident.

Comparison with developing countries

Although Russia is not a developing country, there are similarities and differences between the Russian situation

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and that of developing countries. Developing countries tend to be characterized by insufficient data, in terms of both quantity and quality resulting from low numbers of observational sites and poor quality of the acquired data. Russia has a very large database; however, it is problematic in terms of quality. In addition, Russia's geographical size (the area of the Russian Federation is 17 075 400 km^2, which is 70% of the territory of the former U.S.S.R. or 16 905 400 km^2 without the Arctic Ocean islands) is frequently incomparable with the size of most developing countries and, consequently, also the scale of related problems. Although the main sources of anthropogenic impact on surface waters are concentrated in the European part of the Russian Federation (population density is 49–81 persons·km^{-2}), other territories — poorly accessible areas of Siberia and the Far East (population density <1 person·km^{-2}) — are also prone to deterioration of their surface water quality due to the effects of wastes from metallurgy plants, coal-burning electric power stations, concrete- and oil-processing plants, coal mining, and cellulose, paper-producing, and chemical enterprises as well as extensive and inefficient use of natural resources (such as iron, magnesium, copper, oil, mercury, chromium, nickel, tungsten, manganese, oil, gas, timber, and valuable fish).

Developing countries are typically characterized by a relatively low level of scientific understanding and technical infrastructure and, as a consequence, welcome foreign technical assistance. In previous times, Russia had an excellent technical infrastructure and world-class knowledge of aquatic science. However, these have eroded so badly in the past decade that many Russian laboratories are now worse than many found in developing countries. Also, the scientific knowledge base on water quality within Russia has also badly deteriorated in comparison with western countries with the result that much of the knowledge needed to modernize its water quality programs must now be imported from western countries. However, Russia also suffers from the delusion within many of its technical organizations that they still retain technical excellence when, in fact, much of the technical expertise has vanished due to economic difficulties. At this time, there is nearly no capability within organizations such as Roshydromet for modern environmental chemistry and toxicology as these are now practised in the west within monitoring programs. Unlike developing countries, Russia could rapidly regain its technical superiority, but only if existing organizations such as Roshydromet are prepared to deal realistically with the current vacuum of technical leadership and use the expertise within Russian academic institutions and from western technical organizations.

Concluding remarks

The national water quality monitoring program operated by Roshydromet is premised on the understanding that environmental protection should be based on state-of-the-art information. Nevertheless, like many large bureaucracies, internal resistance to change resulted in an institutional perspective in which the recognition of important environmental issues became increasingly divergent from the focus on collecting long-term data. For water quality monitoring, the mandate to operate national data programs was implemented within the narrow perspective of a standardized, descriptive program that served a limited national purpose but was not useful for most water quality management. In more recent years, partly due to budget constraints, a failure to look beyond its own narrow vision of monitoring and undertake critical self-analysis of its programs, and an increasing scientific and technical gap between its practice and that of other similar organizations in the west, the organization displayed a variety of systemic failures that have rendered much of its water quality program unreliable, out-of-date, inefficient, and, to a considerable extent, irrelevant to most modern water quality management issues. The consequence is that Roshydromet cannot meet its mandate or provide reliable information now needed for routine decision-making on water quality management.

The monitoring agency and the responsible agency (Roshydromet) were created under different sociopolitical conditions, in which effectiveness was one of the least important issues in the hierarchy of political priorities. Information on the condition of the environment was "closed" and monopolistic and could not be used even for critical analysis inside the federal system. This isolation has resulted in an organization that produces very little information of use in making surface water quality management decisions. Water monitoring and management were not connected, and this situation remains largely unchanged. Unfortunately, it will take more to improve the GSN system in the Russian Federation than a mere reproduction of monitoring systems used in other countries.

Given the size of the Russian landmass, the fact that it suffers from a full range of environmental problems and has national obligations under international treaties and other multilateral arrangements, a monitoring program that addresses issues is vital to the nation's interests. The program should be cost-effective, transparent, accessible, and flexible. In conclusion, there are several key recommendations for change that apply to the Russian system of monitoring: (*i*) chronic underfunding will have to be solved before there will be any meaningful progress, (*ii*) complete rethinking of the program is necessary so that it reflects the needs of identified users of data and is operated within a sustainable and economic manner, (*iii*) new technical leadership is essential to ensure that appropriate changes are made to the system, (*iv*) the existing database needs to be purged of incorrect and demonstrably false data, (*v*) access to data needs to be improved, and (*vi*) transparency of operations is essential.

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Is the sampling strategy interfering with the study of spatial variability of zooplankton communities?

Carol Avois, Pierre Legendre, Stéphane Masson, and Bernadette Pinel-Alloul

Abstract: Surveys at the whole-lake scale take some time to carry out: several hours or several days. For logistic reasons, the sites are not sampled simultaneously or in a random sequence. Traditional limnological sampling methods require an appreciable amount of time at each site. Any sampling strategy that is not random or simultaneous introduces dependencies among the observations, which must be taken into account during the analysis and interpretation of the data. What is the real nature of the variation measured using a given sampling design? This question is approached using sites sampled by two boat teams during two consecutive days. Statistical modelling was used to partition the variation of zooplankton size-class data into environmental and spatial components. The conclusions reached after an analysis that did not control for the sampling design are erroneous and quite different from those reached when the effect of the sampling design (factors Day, Boat, and Hour) was taken into account. Clearly, when a significant effect of the sampling design is found, one must control for it during the analysis and interpretation of ecological variation.

Résumé : Réaliser un relevé à l'échelle panlacustre peut prendre du temps : plusieurs heures ou même plusieurs jours. Pour des raisons de logistique, les stations ne sont pas échantillonnées simultanément ni en ordre aléatoire Les méthodes classiques d'échantillonnage limnologique imposent de consacrer un temps appréciable à chaque station. Une stratégie d'échantillonnage qui n'est ni simultanée ni aléatoire introduit des dépendances entre les observations, ce qui doit être pris en compte dans l'analyse et l'interprétation des données. Quelle est la vraie nature de la variation mesurée avec un plan d'échantillonnage donné? Nous avons abordé la question en examinant des stations échantillonnées par deux équipes embarquées pendant deux journées consécutives. La modélisation statistique a servi à répartir la variation des données sur les classes de taille du zooplancton en composantes environnementales et spatiales. Les conclusions atteintes après une analyse qui ne tenait pas compte du plan d'échantillonnage sont erronées et très différentes de celles que l'on obtient quand on prend en compte l'effet du plan d'échantillonnage (les facteurs jour, bateau et heure). Il apparaît clairement que, si l'on observe un effet significatif du plan d'échantillonnage, il faut le prendre en compte dans l'analyse et l'interprétation de la variation écologique.

[Traduit par la Rédaction]

Introduction

Studies on the spatial variation of ecological communities began at the turn of century. Perception of the importance of spatial structures has changed with time. Whereas spatial variation was considered a statistical nuisance some years back (e.g., Steele 1976), it is now recognised as an ecologically important feature of ecosystems (Platt and Harrison 1985; Legendre 1993). Concomitant with this new approach is the notion that the patterns of spatial variation to be observed may depend on the scale of observation (e.g., Levin 1992; Legendre et al. 1997). Methods have been developed for analysing spatial variation as an ecological quantity, at a single scale or as a function of scales (e.g., Dutilleul 1998; Gardner 1998). The success of such analyses depends, however, on hypotheses and on sampling strategies; in a great many cases, sampling strategies are decided a priori without studying their possible effects on the analysis of the ecological phenomenon.

Many studies of freshwater zooplankton have considered the spatial heterogeneity of community composition along the vertical axis. The sampling strategies used in these studies assumed that the habitat was unstructured horizontally, the distribution of zooplankton being homogeneous or with negligible variance compared with the vertical axis (Keller and Yan 1991; Pinel-Alloul et al. 1995). So sampling was often conducted at a single site in the central portions of water bodies. This view is justified by the work of Richerson et al. (1978), who noted that thermal stratification represents the most important physical characteristic in lakes; it is at the origin of the physical, and hence the biological, heterogeneity among strata. The inadequacy of this sampling strategy to allow for the analysis of the spatial structure of communities was noted (e.g., Malone and McQueen 1983) when limnologists became interested in horizontal spatial variation of zooplankton at different scales of observation (e.g., Pinel-Alloul and Pont 1991; Lacroix and Lescher-Moutoué 1995). Few authors have simultaneously studied the spatial variation of zooplankton along the horizontal and vertical axes as well as the interaction between these axes (e.g., Pinel-Alloul and Pont 1991; Masson and Pinel-Alloul 1998). Many investigations pointed out the importance of the sampling design when studying the spatial structure of

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communities and the environmental variables that may influence them (e.g., Pinel-Alloul and Pont 1991). The spatial distribution of zooplankton depends to a large extent on the observation scale; spatial heterogeneity increases rapidly with the extent of the sampling area, and communities have different types of spatial distribution at different scales (e.g., Pinel-Alloul and Pont 1991; Lacroix and Lescher-Moutoué 1995). Patalas and Salki (1993) insisted on the fact that the type of spatial structure that can be detected in lake communities depends on the size of the lake (i.e., the "extent" of the sampling area) and on the number of sampling sites (which, together with the extent, determines the sampling interval; the components of a scale are described by Legendre and Legendre 1998).

Zooplankton patches have been detected on scales ranging from centimetres (1–100 cm; Byron et al. 1983; Butorina 1986) to kilometres (1–100 km; Richerson et al. 1978; Patalas and Salki 1992), but the greatest spatial heterogeneity was observed along the vertical rather than the horizontal axis. Spatial variation in plankton distributions in whole lakes has been attributed to a wide variety of environmental factors (Lens et al. 1986). The environmental variables that explain the spatial structure of zooplankton communities also depend on the scale of the observations. Malone and McQueen (1983) recognised four categories of spatial structures representing: (*i*) large patterns (>1 km in diameter) induced by vectorial forces (Patalas 1969) or by seasonal (Urabe 1989) and morphometric variables (Urabe and Murano 1986), (*ii*) coarse- and fine-scaled patterns (approximately 10–1000 m in diameter) caused by wind-induced currents (e.g., Riley 1976) or by the combined effects of shore avoidance and vertical migrations (Ringelberg 1991), (*iii*) Langmuir circulation patterns caused by behavioural interactions (De Nie et al. 1980) and a combination of water movement, light orientation, and active swimming, and (*iv*) swarming patterns (from a few centimetres to several metres in diameter), potentially caused by biotic factors. Zooplankton patchiness over broad and fine scales is the result of many physical and chemical processes interacting with several biological processes, as described by the "multiple driving force hypothesis" (for a review, see Pinel-Alloul 1995), with the predominance of abiotic factors at large spatial scales and that of biotic factors at smaller scales.

Sampling strategies for analysing the horizontal and vertical structures of zooplankton communities have focused, in most cases, on spatially limited lake areas (e.g., transects or quadrats; e.g., Pinel-Alloul and Pont 1991; Visman et al. 1994). Ecologists now wish to maximize the extent of the study area (Masson and Pinel-Alloul 1998) and the number of sites sampled in order to expand the investigations to a scale of observation covering whole lakes (Pinel-Alloul et al. 1999). By doing so, they hope to understand the spatial structuring of communities at that scale and be able to compare the results with the spatial structures determined for spatially limited lake areas (Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). However, this may change the nature of the variation measured, since, generally, all sites are not sampled simultaneously. In many studies where sampling covered a few hours or days, the temporal effects were not taken into account during the analysis of spatial variation because daily and short-term variations were considered negligible (e.g., Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). Was this assumption justified? The effects of the abiotic and biotic factors on the spatial variation of zooplankton change not only with the spatial scale but also with the temporal scale over days or seasons (e.g., Pinel-Alloul and Pont 1991). Gaston and McArdle (1994) recognised the importance of incorporating the temporal dependency of spatial sampling in their analysis.

Temporal and spatiotemporal short-term variations in community structure are well established. They are inherent to the zooplankton community, caused by migrations of organisms which may be vertical and (or) horizontal (Angeli et al. 1995; Lauridsen and Buenk 1996) and linked or not to diurnal variations (e.g., Lauridsen and Buenk 1996). Light (e.g., Ringelberg 1991; Richards et al. 1996), food (Haney and Hall 1975), temperature of the upper water layers (Manca et al. 1986), competition (e.g., Wright et al. 1980), and visual or nonvisual predation (Bollens and Frost 1989; Herwig and Schindler 1996) are all important in explaining migration movements. Some of these factors, like the physical stratification of water columns (Richerson et al. 1978; Pinel-Alloul et al. 1988), predation and competition (e.g., Visman et al. 1994), and sources of food (Tessier 1986), are also considered important in determining the spatial distributions of zooplankton communities (Pinel-Alloul 1995).

An artifactual spatial structure may be generated in the data by the sampling design, caused by the route followed by the boats visiting the sampling stations in sequence, if the duration of the trip is important (Ibanez 1973). In such a case, the sampling design does not guarantee that spatial variation is the only type of variation found in the data, and we must ask the question: what is the real nature of the variation measured using the sampling design?

The original purpose of the sampling program reported in this paper was to verify hypotheses put forward in previous studies at the within-lake scale ($H(1.x)$ designates an alternative hypothesis whereas $H(0.x)$ stands for the corresponding null hypothesis): $H(1.2)$: "the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton" and $H(1.3)$: "a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton"; the sampling extent was the whole lake.

Actually, the sampling strategy used in the field generated variation among sites that may look like spatial variation,while in fact, it may reflect other aspects of the sampling design. To check this, the following questions must be asked. How large is the short-term temporal variation found among sites during sampling compared with the spatial variation? How does the collinearity between short-term temporal variation and spatial variation affect the relationships identified between the zooplankton and the abiotic and biotic factors? Accordingly, the objectives of this paper are (*i*) to test the null hypothesis $H(0.1)$ that "there is no significant effect of the sampling design on the detected spatial structure of the zooplankton data" and (*ii*) if a significant effect is identified ($H(1.1)$) to determine its impact on the analysis of the spatial structure of the zooplankton data, before proceeding with hypotheses $H(1.2)$ and $H(1.3)$ above. On the other hand, if a significant effect of the sampling design is not identified, one can proceed directly with hypotheses $H(1.2)$ and $H(1.3)$.

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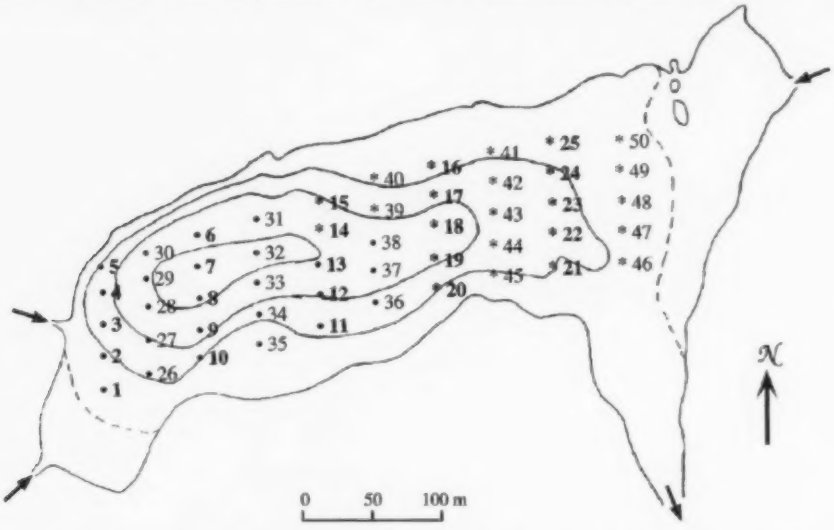
Fig. 1. Map of Lake Cromwell (74°00′W, 45°49′N) showing the 50 sampling sites in Québec, Qué. Day 1 (bold): sites 1–25; day 2: sites 26–50. The sites sampled from boats 1 and 2 are identified by a bullet and an asterisk, respectively. Solid lines are the isobaths (3, 6, and 9 m), broken lines delimit the macrophyte zones, and arrows represent water inputs and output.



The statistical methods used to test these hypotheses are appropriate for lakes; they may also prove useful in studies of marine plankton. The description of the spatial distribution of zooplankton and its interpretation using explanatory abiotic and biotic factors (hypotheses $H(1.2)$ and $H(1.3)$ above) will be the subject of another paper.

Materials and methods

Sampling strategy

Lake Cromwell (74°00′W, 45°49′N) is located on the territory of the Station de biologie des Laurentides (Université de Montréal), approximately 80 km north of Montréal, Québec. This small Canadian Shield mesotrophic lake (surface area 9.2 ha) is shallow (mean and maximum depths of 3 and 9.1 m, respectively), humic, lightly acidic, and dimictic (Pinel-Alloul and Pont 1991). Lake Cromwell is also characterised by high planktivorous and benthivorous fish abundances and the absence of piscivorous fish species.

Considering the purposes of this study as well as the results of previous investigations on the spatial structure of zooplankton, particularly those conducted previously in Lake Cromwell (e.g., Pinel-Alloul and Pont 1991), a sampling strategy was developed to cover the whole lake while minimising the variance due to the temporal variation inherent to zooplankton communities. Sampling, conducted in the summer, was diurnal (from 07:00 to 19:00) to eliminate possible effects of nycthemeral vertical migrations of zooplankton. The lake area, diurnal period, and allowable sampling effort were considered in determining the elements of the sampling scale (grain size, extent, and sampling interval). Systematic sampling of 50 sites, in a staggered arrangement on the surface of the lake, was conducted during 2 consecutive days (factor Day below); 25 sites were sampled on 3 August (sites 1–25) and 25 more on 4 August 1994 (sites 26–50). Two teams (hereafter called "boats") were at work each day, conducting sampling at the first 13 sites (out of 25) in parallel with the last 12 sites in order to reduce possible daily effects (Fig. 1). The time of sampling was recorded for each site (factor Hour below). The spatial sampling interval was 35 m east–west and 22 m north–south; so the sampling grid allowed the study of coarse-scale spatial phenomena (type II variation in Pinel-Alloul and Pont 1991). Due to lake morphology, the limnetic strata contained 50 sampling points in the epilimnion (0–2.5 m), 36 in the metalimnion (2.5–5 m), and 13 in the hypolimnion (5 m-bottom). The sampling dates were chosen to correspond to the period of maximum summer stratification (Jordan et al. 1988).

At each site, 11 physical and chemical variables were measured: pH, turbidity (NTU), temperature (degrees Celsius), alkalinity (milligrams per litre), maximum depth (metres), conductivity (microsiemens per centimetre), dissolved oxygen (DO, milligrams per litre), dissolved silica (DSI, micrograms per litre), total dissolved nitrogen (TDN, micrograms per litre), dissolved organic carbon (DOC, milligrams per litre), and total dissolved phosphorus (TDP, micrograms per litre). Four biological variables were also measured: chlorophyll *a* concentration (micrograms per litre) of phytoplankton <20 and >20 μm, density (individuals per litre) and mean length (millimetres) of Chaoboridae. The biomass of limnoplankton (micrograms per litre) (i.e., all zooplankton and organic matter collected in the water samples) was the response variable of the study; it was divided into four size-classes (>500, 202–500, 100–202, and 53–100 μm). The field and laboratory methods are fully described in Masson and Pinel-Alloul (1998).

Statistical analyses

Is there a significant effect of the sampling design on the detected spatial structure of the zooplankton size-class data?

Multifactorial analysis of covariance (ANCOVA) was used to test the hypothesis $H(0.1)$ of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data. Multifactorial analyses of variance (ANOVA) and covariance (ANCOVA) are tools which provide insights into the nature of variation of natural events, considering simultaneously the effects of several factors. Three factors were used to characterise the sampling design: Day (two levels: fixed and qualitative; this factor is considered fixed because the sampling days were chosen to be consecutive), Boat (two levels: fixed and qualitative), and Hour (covariable). The factors Day and Boat are crossed. The design of

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Table 1. Three-way ANCOVA design with two fixed factors and one random factor (mixed model) used to test the hypothesis $H(0.1)$ of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data. MS = mean square.

Source of variation	MS	F statistic
Main effects		
Day (d), fixed, qualitative, balanced	MS_d	MS_d/MS_{hd}
Boat (b), fixed, qualitative, unbalanced	MS_b	MS_b/MS_{hb}
Hour (h), covariable	MS_h	MS_h/MS_{res}
First-order interactions		
Day × Boat (db)	MS_{db}	MS_{db}/MS_{dbh}
Hour × Day (hd)	MS_{hd}	MS_{hd}/MS_{res}
Hour × Boat (hb)	MS_{hb}	MS_{hb}/MS_{res}
Second-order interaction		
Day × Boat × Hour (dbh)	MS_{dbh}	MS_{dbh}/MS_{res}
Residual (res)	MS_{res}	

the analysis was unbalanced, since the factor Boat had unequal sample sizes (i.e., level 1, $n = 13$; level 2, $n = 12$), so type III sums of squares (SS) were used in the analyses (Shaw and Mitchell-Olds 1993). Type III SS are designed to quantify the effects of particular factors adjusted for all the other factors in the model; they have the property that the various SS resulting from the partition do not add up to the total SS. The hypotheses tested using type III SS are constructed as if each combination of factors in the model had an equal number of observations, giving each combination of factors an equal weight. Thus, three-way ANCOVA with mixed model, unbalanced, has been used to test the null hypotheses about the main effects Day, Boat, and Hour, the first-order interactions Day × Boat, Hour × Day, and Hour × Boat, and the second-order interaction Day × Boat × Hour in the model (Table 1), starting with the latter: $H_{0(1)}$: there is no interaction between factors Day, Boat, and Hour.

If the second-order interaction was not significant, it was eliminated from the model and the first-order interactions were tested: $H_{0(2)}$: there is no interaction between factors Day and Boat, $H_{0(3)}$: there is no interaction between factors Day and Hour, and $H_{0(4)}$: there is no interaction between factors Boat and Hour.

If there is independence between any effects of Day, Boat, and Hour, the main effects can be tested: $H_{0(5)}$: there are no differences among the levels of factor Day, $H_{0(6)}$: there are no differences among the levels of factor Boat, and $H_{0(7)}$: there are no differences among the levels of factor Hour.

The procedure was stopped when at least one high-order interaction was found to be significant, considering the hierarchy of the analysis. A significant interaction tells us that the size and (or) nature of a main effect changes across the levels of the other factor (and vice versa). Thus, one always examines the highest order interaction first, i.e., Day × Boat × Hour. If this interaction is significant, there is no point in examining the first-order interactions or the main effects on their own. The SS component Day SS measured the unevenness between days, independent of boat and hour (if the interaction terms are not significant). If a difference between days was found, it could be attributed either to temporal variation inherent to the zooplankton size-class data or to the sampling design, since the sites sampled on the two days were neither the same nor paired into strata. The same reasoning applied to the component Hour SS. For the component Boat SS associated with differences between boats, independent of day and hour (if the interaction terms are not significant), the observed variation may have been caused by the routes of the boats, which may have generated a spatial artefact; it may depend on the generation time or the ability of the organisms to move, relative to the duration of the sampling campaign.

It is not possible to determine exactly the cause of the variation explained by the interaction terms. This variation may be caused by factors influencing the migration of organisms (i.e., temporal and (or) spatiotemporal variations of community) or it may be an artefact due to the sampling design. For this reason, we prefer to talk about an "effect of the sampling design," which includes both.

All hypotheses were tested in a single ANCOVA for each size-class of zooplankton. This analysis was done separately for the epi-, meta-, and hypolimnion. The analysis was repeated while considering the three limnetic strata as an additional factor.

We were interested in describing in some detail the effect of the sampling design on the observed patterns of distribution of the zooplankton size-class data in order to optimise the sampling strategy for future studies. A general model was established for the zooplankton community (all four size-classes) in each water stratum and another one for all strata. To obtain a general model, particular models were first computed for each size-class of zooplankton. For each source of variation, the SS from the particular models were added; we recomputed the mean squares, the F ratio (which is then a "stacked" univariate F statistic, as in Verdonschot and ter Braak 1994), and the associated p value, reconstructing the ANCOVA table. The resulting probabilities were approximate, since the covariances among size-classes of zooplankton were not taken into account when summing the SS to create the stacked F statistics; this should not generate any important effect, since the covariances among size-classes were low. Results of the stacked F analysis were verified using redundancy analysis (see below), which is a multivariate method. Therefore, there were as many general models as there were particular models. The general model selected for the zooplankton size-class data in each stratum and for all strata was the one that had the highest-order significant interaction term, provided that the model was significant; this model also had the highest amount of explained variation. If there was no significant interaction term, we selected the model where the significant main effects explained the largest fraction of the total variance. The SS explained by the model represented the variation generated by the "sampling design"; the residual SS was that unexplained by the design.

No significant effect of the sampling design on the detected zooplankton spatial structure

When the null hypothesis $H(0.1)$ could not be rejected, the sampling design was assumed to have no significant effect on the spatial structure of zooplankton and hypotheses $H(0.2)$ and $H(0.3)$ could now be tested. The method of partition of ecological variation between environmental and spatial components (Borcard et al. 1992; Borcard and Legendre 1994) was used for this purpose. The analysis produced four independent and additive fractions of the variation: (E), a fraction attributed to the nonspatially structured component of the environmental variables (abiotic and (or) biotic); (SE), a fraction explained by the spatially structured portion of these explanatory variables; (S), a pure broad-scale spatial component that remains unexplained by the current environmental variables and may reflect processes generating spatial heterogeneity that have not been explicitly included in the analysis; and (Un), a fraction of the ecological variation that remains unexplained by the spatial and environmental variables (Fig. 2*a*). This method produces measures of the importance, for the zooplankton data, of the environmental variables and the broad-scale spatial structure. This analysis was carried out using redundancy analysis (RDA) (Rao 1964, 1973; van den Wollenberg 1977) and partial RDA (ter Braak 1988).

RDA combines the properties of two families of methods: regression and ordination. The steps of RDA are as follows: (1) regress each variable in the matrix of response variables on all the explanatory variables and compute the fitted values and (2) carry

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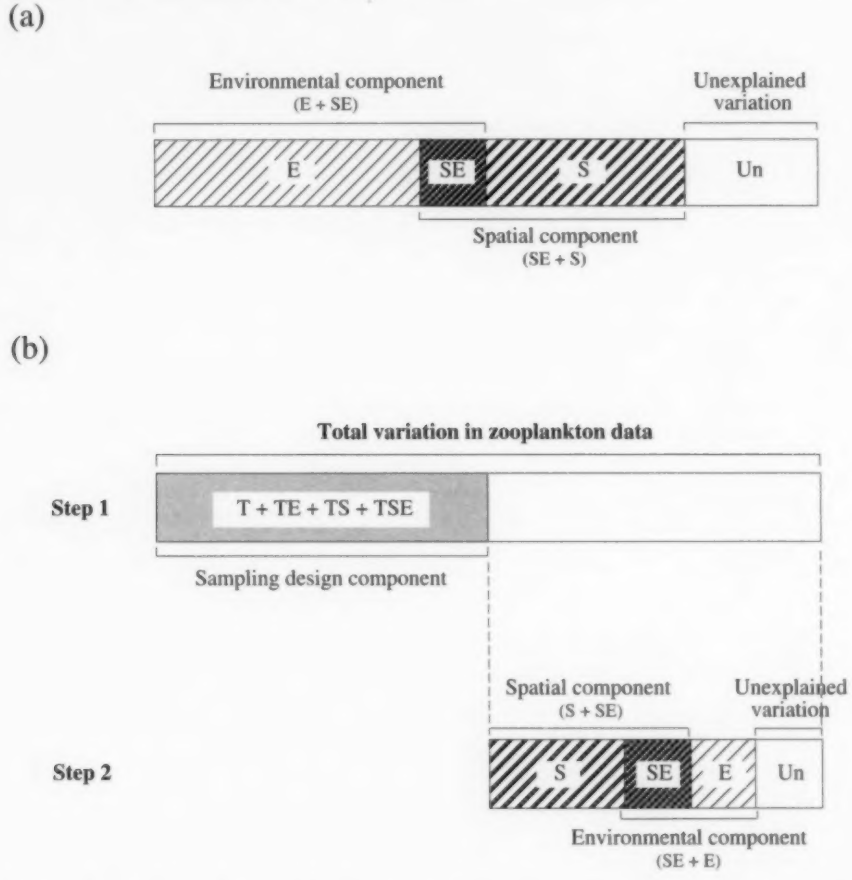
Fig. 2. (*a*) Partition of the ecological variation of the zooplankton data following Borcard et al. (1992). The total rectangle represents the ecological variation. It is divided into the following: (E), pure environmental variation; (SE), spatially structured environmental variation; (S), pure spatial variation; (Un), unexplained variation. (*b*) Partition of total variation of the zooplankton data, including the sampling design. Step 1: quantification and elimination of the variation explained by the sampling design, which includes the following: (T), pure sampling design variation; (TE), environmental variation explained by the sampling design; (TS), spatial variation explained by the sampling design; (TSE), spatially structured environmental variation explained by the sampling design. Step 2: partition of the residual variation among the environmental and spatial components.



out a principal components analysis of the matrix of fitted values to obtain the eigenvalues and eigenvectors representing the variation explained by the explanatory variables. Partial RDA is a direct extension of partial regression (for details, see Legendre and Legendre 1998). Using RDA, fractions (S + SE + E), (S + SE), and (SE + E) were determined; fractions (S) and (E) were obtained by partial RDA, whereas fraction (SE) was obtained by difference, since (SE) = (S + SE) + (SE + E) – (S + SE + E) (Fig. 2*a*).

Significant effect of the sampling design on the detected zooplankton spatial structure

Rejecting hypothesis $H(0.1)$ meant that the sampling design had a significant effect on the spatial structure of the zooplankton variables. When this was the case, the variation of the zooplankton size-class data was analysed in more detail, considering all the deterministic components of the variation for which information was available (Fig. 2*b*). (*i*) The sampling design component (T) characterises the variation generated by the sampling program. This component contains the factors Day, Boat, and Hour as well as their interaction terms and includes (T), (TE), (TS), and (TSE) as defined in the caption to Fig. 2. (*ii*) The spatial component (S) characterises the spatial structure (i.e., the spatial distribution of organisms in their habitat) and is modelled using the geographic positions of the sites, as described below. Fractions (S), (SE), (TS), and (TSE) of variation contain this component. (*iii*) The environmental component (E) includes the effects of abiotic and biotic variables. This component characterises the organisational (functional) structure of populations or communities (reproduction, growth, density, mortality, etc.). Fractions (E), (SE), (TE), and (TSE) of variation contain parts of this component.

Although we cannot be certain whether the components (TE), (TS), and (TSE) should be attributed to the sampling design or to spatial or environmental effects, conservative interpretation of the results requires that we remove all the variance that can be attributed to the sampling design, i.e., fractions (T), (TE), (TS), and (TSE), before testing ecological hypotheses $H(1.2)$ and $H(1.3)$ (Fig. 2*b*). By doing this, we place ourselves in a situation less likely to promote our hypotheses. This is in agreement with the principle of parsimony (Ockham's razor): we must first try to attribute the observed variance to the most simple source of varia-

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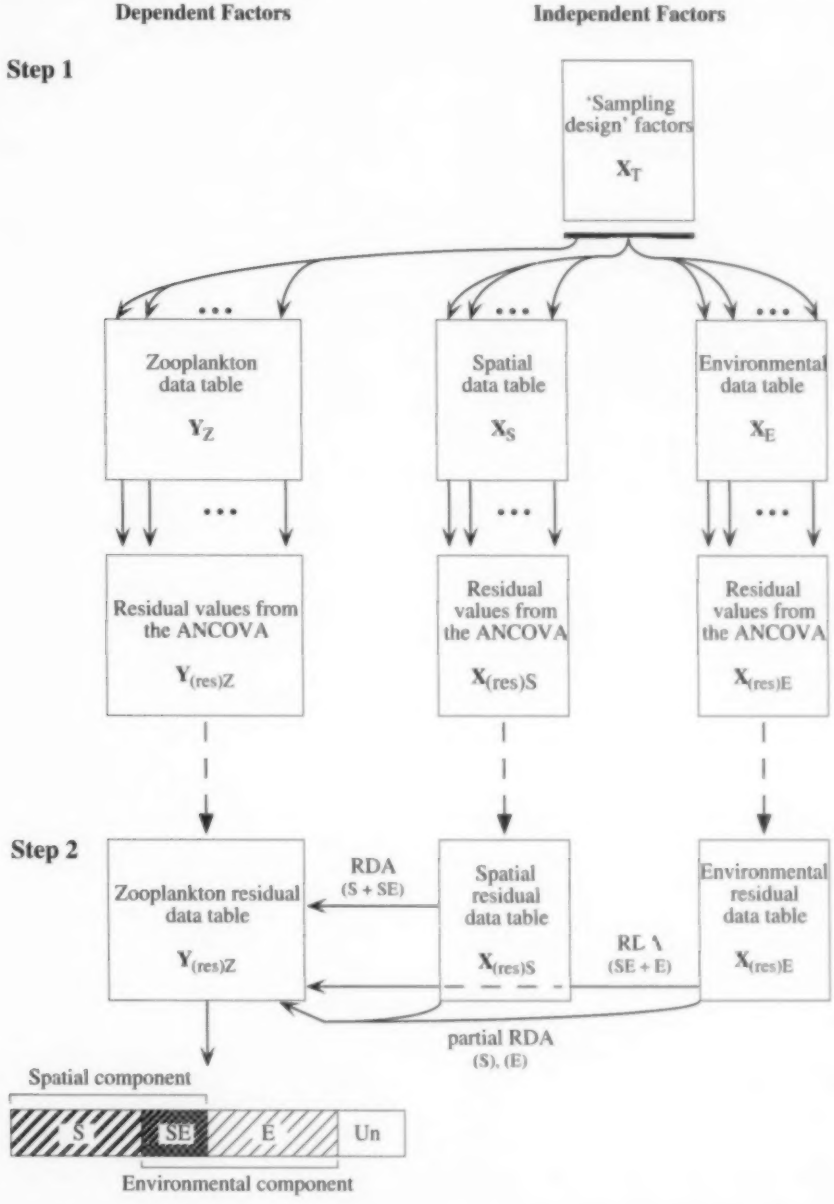
Fig. 3. Summary of the method for partitioning the measured variation among the spatial and environmental components while controlling for the effect of the sampling design (method 1). Step 1: ANCOVA for each variable y_Z, x_S, and x_E with the sampling design factors of the general model $\mathbf{X}_T$. Compute the residual values $y_{(res)Z}$, $x_{(res)S}$, and $x_{(res)E}$ and construct the residual tables $\mathbf{Y}_{(res)Z}$, $\mathbf{X}_{(res)S}$, and $\mathbf{X}_{(res)E}$ in view of the second step. Step 2: RDA and partial RDA to partition the residual variation of zooplankton among the spatial and environmental variables.



tion, which is the sampling design in the present study. In the most extreme case, it would account for all the variation of the response variables, leaving nothing to be explained by our ecological hypotheses.

The first step of the analysis was to find the ANCOVA residuals representing the variation unexplained by the factors of the sampling design for each zooplankton size-class variable, thus eliminating the fraction (T + TE + TS + TSE) of Fig. 2*b*. The table of residuals was constructed (Fig. 3) and became the new matrix of response variables for the second step of the analysis. Before proceeding with the variance partitioning, it was necessary to also eliminate the effect of the sampling design from the deterministic components. An ANCOVA against the sampling design factors was carried out for each explanatory variable, and residual values were computed; they were assembled into tables of residuals of the environmental and spatial variables. In the second step of the anal-

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Table 2. Summary of analyses for partitioning the measured variation to obtain fractions (T), (S), (E), (SE), (TE), (TS), and (TSE) where (T), (S), and (E) are sampling design, spatial, and environmental components, respectively.

Type of analysis	Matrix of explanatory variables	Matrix of covariables	Measured fraction
RDA (1)	Sampling design		(T + TE + TS + TSE)
Partial RDA (2)	Environmental (forward selection)	Sampling design	(E + SE)
Partial RDA (3)	Spatial (forward selection)	Sampling design	(S + SE)
Partial RDA	Environmental and spatial	Sampling design	(S + SE + E)
Partial RDA	Sampling design and spatial	Environmental	(T + TS + S)
Partial RDA	Sampling design and environmental	Spatial	(T + TE + E)
Partial RDA	Sampling design	Environmental and spatial	(T)
Partial RDA	Environmental	Sampling design and spatial	(E)
Partial RDA	Spatial	Sampling design and environmental	(S)

Note: Fractions (T), (S), and (E) are obtained directly from the analyses, whereas fractions (SE), (TE), (TS), and (TSE) are calculated by difference: (SE) = (S + SE) + (E + SE) − (S + SE + E), (TE) = (T + TE + E) − (T) − (E), (TS) = (T + TS + S) − (T) − (S), and (TSE) = (T + TE + TS + TSE) − (T) − (TE) − (TS) or (T + TE + TS + TSE) − (T + TE + E) − (T + TS + S) + (T) + (E) + (S). The first three steps are carried out in the order indicated (1, 2, 3), including a forward-selection procedure for steps 2 and 3. The sampling design, environmental, and spatial factors are used as explanatory variables and covariables, respectively, in the RDA and partial RDA runs.

ysis (Fig. 3), these new matrices were used as "environmental variables" in RDA and as "covariables" in partial RDA, allowing partitioning of the residual variation of zooplankton among the environmental and spatial components. Steps 1 and 2, as described here and in Fig. 3, form method 1.

RDA was used to verify the results obtained by ANCOVA and the stacked univariate *F* statistic. The factors describing the sampling design in the ANCOVA were encoded and formed the matrix of explanatory variables in RDA. The qualitative factors Day and Boat were binary coded (−1, 1); the number of dummy variables necessary to code for a factor is equal to the number of levels minus 1. The quantitative factor Hour (i.e., the covariable of the ANCOVA) was not recoded. The interaction terms were obtained by multiplying the dummy variables coding for the factors involved in the interaction. The number of dummy variables necessary to code for a factor or an interaction term is equal to the number of its degrees of freedom. We obtained the fraction (T + TE + TS + TSE) of the variation explained by the sampling design factors and compared it with the results of the combination of ANCOVA and the stacked univariate *F* statistic. We also used the matrix of sampling design factors as the matrix of covariables in partial RDA runs, in which the environmental and spatial variables, in turn, formed the matrices of explanatory variables. This procedure, called method 2, allowed us to verify the selection of environmental and spatial variables made by the variation partitioning method after controlling for the effect of the sampling design and to quantify the variation generated exclusively by the sampling design (i.e., fraction (T)). Using these results, all the fractions of variation for the available variables could now be determined: (T), (TE), (TS), (TSE), (S), (SE), and (E) (see Table 2). Tests of equality of variances (Sokal and Rohlf 1995) were computed to compare the variances among the four size-classes in each stratum, the variances among the three strata for the total biomass of zooplankton, and the variances among size-classes for the three strata using stacked univariate *F* statistics. The probabilities were examined after Bonferroni correction (Legendre and Legendre 1998).

Practical aspects of the statistical analyses

Before undertaking the analyses, histograms of frequency distributions were produced for all measured variables; a suitable normalisation was calculated for all variables that were not normally or symmetrically distributed using the Kolmogorov–Smirnov test of normality (using the tables corrected by Lilliefors 1967). All phytoplankton and zooplankton variables were normalised by logarithmic transformation, $\log(100x)$; the constant 100 was used in order to prevent the appearance of negative transformed values. The transformed data were used in all subsequent statistical analyses.

The matrix of response variables contained the transformed biomasses of the four zooplankton size-classes. The matrix of spatial variables was constructed using a polynomial of the Cartesian coordinates (*X*, *Y*) of the sites, as suggested by Legendre (1990). The forward-selection procedure of program CANOCO was used to select the terms of the geographic polynomial that significantly contributed to the explanation of the zooplankton size-classes. In all analyses, the polynomial of degree 4, made of 14 spatial monomials, was used as the starting point of the backward-selection procedure.

Before selecting the environmental variables (abiotic and biotic) to be used in RDA and partial RDA, it was necessary to establish the type of relationship found between the response and explanatory variables. Pearson correlation coefficients combined with scatter diagrams allowed us to determine if the relationships between variables of the two groups were linear; in the case of nonlinear relationships, a polynomial of the environmental variables (containing the centred variables to the powers 1 and 2) was used. Following the forward selection of variables, using in each case the power(s) explaining the largest amount of variation, 20 physical and chemical variables and two morphometric variables were retained to form the matrix of abiotic variables.

While depth is designated as a morphometric variable, it may also be an indicator (proxy variable) for the spatial distribution of fish in the lake; this biological variable is pertinent when studying the spatial distribution of zooplankton size-classes. Fish echolocation data obtained by echosounder in Lake Cromwell during the summer of 1994 (Gaudreau and Boisclair 1998) showed a spatial distribution of fish with low abundances of fish in the pelagic zone and high abundances in the littoral zone.

The matrix of biotic variables contained two size-classes of phytoplankton as well as the density and mean length of *Chaoborus* larvae. The size fraction <20 μm represents the edible algae, an important food resource for zooplankton. Depletion of edible algae biomass is generally related to strong zooplankton grazing (e.g., Angeli et al. 1995). The size fraction >20 μm represents the inedible algae; in this fraction, filamentous cyanobacteria may inhibit zooplankton grazing by mechanical interference or toxic effect (Ghadouani et al. 1998). Invertebrate predation by chaoborid larvae zooplankton is also an important factor controlling the zooplankton biomass, especially in humid or fishless lakes (Masson and Pinel-Alloul 1998). *Chaoborus* predation on zoo-

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Table 3. ANCOVA tables to test the hypothesis $H(0.1)$ of no significant effect of the sampling design on the zooplankton spatial structure for all dependent variables (i.e., the four size-classes of zooplankton), defining a general model for each stratum. MS = mean square.

General model of zooplankton per stratum	Source of variation	df	Type III SS	MS	F statistic
Epilimnion	Day	1	1.3416	1.3416	0.7544 ns
	Boat	1	2.3996	2.3996	1.1221 ns
	Hour	1	0.0768	0.0768	0.4717 ns
	Hour × Day	1	1.7783	1.7783	10.9232**
	Hour × Boat	1	2.1384	2.1384	13.1351***
	Model	5	5.3745	1.0749	6.6026***
	Residual	44	7.1646	0.1628	
	Total	49	12.5391		
Metalimnion	Boat	1	5.7073	5.7073	20.0820***
	Model	1	5.7073	5.7073	20.0820***
	Residual	34	9.6637	0.2842	
	Total	35	15.3709		

Note: Significant F statistics are coded as follows: ***$p \leq 0.001$; **$p \leq 0.01$; ns, not significant.

plankton appears to be controlled by both density and size of predators (e.g., Pinel-Alloul 1995).

The limnetic strata were coded in the form of two (–1, 1) dummy variables; these variables, as well as their interactions with the X and Y spatial coordinates, were included among the spatial variables in the study of the vertical spatial distribution of zooplankton. These dummy variables were considered to represent a physical forcing variable, since stratification of the water column is generated by temperature and may induce spatial heterogeneity of the zooplankton (Richerson et al. 1978); if this was the case, we expected a strong correlation between temperature and the dummy "strata" variables. The limnetic strata may also been considered as representing spatial variables, since they code for the coordinates of the sampling points along the vertical axis. We also included in the analysis the interactions between the spatial monomials describing the horizontal distribution of the sites and the vertical limnetic strata.

To select the explanatory variables (environmental and spatial) that significantly explained the ecological variation of zooplankton and to partition this variation, we used the forward-selection procedure available in the program CANOCO for RDA. Environmental variables were selected independently of the spatial variables. The selected variables determined the fractions (E + SE) and (S + SE), respectively. We then analysed all selected variables together to determine the fraction (S + SE + E). Using the three quantities (E + SE), (S + SE), and (S + SE + E), the fractions (S), (SE), and (E) were obtained; note that fractions (E) and (S) could also have been obtained directly using partial RDA (Borcard and Legendre 1994). The contribution of each selected variable and that of the whole set of variables were tested by permutation under the full model (ter Braak 1990). The statistical analyses were first carried out along the horizontal axes of the lake (i.e., for the epi-, meta-, and hypolimnion separately) and then along the vertical axis.

ANOVAs and ANCOVAs were carried out using SuperANOVA™ version 1.11. The program CANOCO™ version 3.11 (ter Braak 1990) was used for the RDA and partial RDA.

Results

Is there a significant effect of the sampling design on the detected spatial structure of the zooplankton size-class data?

ANCOVA was carried out to test the null hypothesis $H(0.1)$ of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data along the horizontal axis, i.e., in the epi-, meta-, and hypolimnion separately, and vertically, i.e., across the three strata.

Horizontal spatial structure

The null hypothesis was rejected for the epi- and metalimnion but not for the hypolimnion. As the null hypothesis $H(0.1)$ could not be rejected for the hypolimnion, the null hypothesis $H(0.2)$ corresponding to hypothesis $H(1.2)$ that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton was tested using the method of partition of ecological variation of Borcard et al. (1992) (see below).

As a significant effect of the sampling design is identified for the epi- and metalimnion, the variation of the zooplankton size-class data was analysed considering the sampling design component. For these two strata, the general models established after ANCOVA and the stacked univariate F statistics show a significant variation generated by the sampling design variables; the coefficients of determination (R^2) were 0.4286 ($p < 0.001$) for the epilimnion and 0.3713 ($p < 0.001$) for the metalimnion (Table 3).

In the epilimnion, the variation generated by the sampling design variables was attributed in great part to the interaction terms Hour × Day and Hour × Boat ($R^2 = 0.1418$, $p < 0.01$ and $R^2 = 0.1705$, $p < 0.001$, respectively). This indicated that factors Hour and Day on the one hand and Hour and Boat on the other hand were not independent and that the biomass of zooplankton changed as a function of these effects. For each first-order interaction term, two different effects were found: Hour × Day 1 and Hour × Day 2 for the interaction Hour × Day and Hour × Boat 1 and Hour × Boat 2 for the interaction Hour × Boat; for each effect, the zooplankton biomass was significantly different. The factor Boat is the only factor that generates significant variation in the metalimnion; it amounts to 37.13% ($p < 0.001$) of the total variation measured in this stratum.

Each size-class of epi- and metalimnetic zooplankton is significantly influenced by the sampling design variables (Tables 4 and 5). In the epilimnion, the particular models of ANCOVA are similar to the general ANCOVA model, ex-

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Table 4. Particular models (ANCOVA tables) for each size-class of epilimnetic zooplankton to test the hypothesis $H(0.1)$ of no significant effect of the sampling design on the zooplankton spatial structure. MS = mean square.

Dependent variable	Source of variation	df	Type III SS	MS	*F* statistic
Zooplanton >500 μm	Day	1	0.1116	0.1116	0.5018 ns
	Boat	1	0.5657	0.5657	1.2419 ns
	Hour	1	0.0001	0.0001	0.0026 ns
	Hour × Day	1	0.2224	0.2224	5.7172*
	Hour × Boat	1	0.4555	0.4555	11.7095***
	Model	5	1.1021	0.2204	5.6658***
	Residual	44	1.7129	0.0389	
	Total	49	2.8150		
Zooplanton 202–500 μm	Day	1	0.8043	0.8043	0.8336 ns
	Boat	1	1.4002	1.4002	1.0168 ns
	Hour	1	0.0004	0.0004	0.0056 ns
	Hour × Day	1	0.9648	0.9648	13.5887***
	Hour × Boat	1	1.3771	1.3771	19.3958***
	Model	5	2.7231	0.5446	7.6704***
	Residual	44	3.1242	0.0710	
	Total	49	5.8474		
Zooplanton 100–202 μm	Day	1	0.3637	0.3637	0.8126 ns
	Boat	1	0.2221	0.2221	7.2238**
	Hour	1	0.0100	0.0100	0.3249 ns
	Hour × Day	1	0.4476	0.4476	14.5602***
	Model	4	0.7071	0.1768	5.7508***
	Residual	45	1.3833	0.0307	
	Total	49	2.0904		
Zooplanton 53–100 μm	Day	1	0.0980	0.0980	0.5054 ns
	Boat	1	0.2563	0.2563	1.1971 ns
	Hour	1	0.0526	0.0526	2.2383 ns
	Hour × Day	1	0.1939	0.1939	8.2511**
	Hour × Boat	1	0.2141	0.2141	9.1106**
	Model	5	0.7504	0.1501	6.3872***
	Residual	44	1.0359	0.0235	
	Total	49	1.7863		

Note: Significant *F* statistics are coded as follows: ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; ns, not significant.

Table 5. Particular models (ANCOVA tables) for the size classes of metalimnetic zooplankton.

Dependent variable	Source of variation	df	Type III SS	MS	*F* statistic
Zooplanton >500 μm	Boat	1	0.9545	0.9545	20.9780***
	Residual	34	1.5480	0.0455	
	Total	35	2.5025		
Zooplanton 202–500 μm	Boat	1	1.7877	1.7877	22.6291***
	Residual	34	2.6844	0.0790	
	Total	35	4.4721		
Zooplanton 100–202 μm	Boat	1	1.5769	1.5769	16.2567***
	Residual	34	3.2985	0.0970	
	Total	35	4.8754		
Zooplanton 53–100 μm	Boat	1	1.3882	1.3882	22.1404***
	Residual	34	2.1328	0.0627	
	Total	35	3.5209		

Note: Significant *F* statistics are coded as follows: ***$p \leq 0.001$; ns, not significant.

cept for size-class 100–202 μm for which the variation is mostly due to the Hour × Day interaction and to the factor Boat ($R^2 = 0.2141$, $p < 0.001$ and $R^2 = 0.1062$, $p < 0.01$, respectively). For the other three size-classes, the first-order interaction Hour × Boat explains the largest fraction of the variation (from 11.99% for size-class 53–100 μm to 23.55% for size-class 202–500 μm) followed by the interaction Hour × Day (from 7.90% for size-class >500 μm to 16.50% for size-class 202–500 μm). The sampling design variation explains from 33.83% of the variance for size-class 100–

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Table 6. Partitioning of the variation measured for each stratum and vertically across the three strata.

	Epilimnion		Metalimnion			
Partition of the variation	Method 1	Method 2	Method 1	Method 2	Hypolimnion	Vertical axis
Total variance	0.2559	0.2559	0.4392	0.4393	0.2834	0.3615
% explained	69.15	69.04	71.55	71.55	75.84	73.36
	0.001	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
(T + TE + TS + TSE)	42.86	42.85	37.13	37.14	na	na
	0.001	*0.001*	*0.001*	*0.001*		
(T)	na	33.39	na	0.42	na	na
		0.001		*0.650*		
(E)	21.89	21.79	28.32	28.32	49.58	8.77
	0.001	*0.001*	*0.001*	*0.001*	*0.001*	*0.121*
(S)	0.43	0.40	3.85	3.84	6.84	11.39
	0.560	*0.560*	*0.046*	*0.027*	*0.119*	*0.049*
(SE)	3.97	4.00	2.25	2.25	19.42	53.20
	0.002	na	*0.005*	na	*0.001*	*0.001*
(TE)	na	3.73	na	11.51	na	na
(TS)	na	12.24	na	–0.04	na	na
(TSE)	na	–6.51	na	25.25	na	na
% unexplained	30.85	30.96	28.45	28.45	24.16	26.64

Note: The variation explained by the fractions (expressed as a percentage; probabilities obtained after 999 permutations are in italics) is indicated for methods 1 and 2 for the epi- and metalimnion. The fractions of variation of the hypolimnion and the vertical axis are obtained directly using the variation partitioning method (Borcard et al. 1992). na, not applicable.

202 μm to 46.57% for size-class 202–500 μm; these fractions are very highly significant ($p < 0.001$) (Table 4). Note that the total variance of size-class 202–500 μm is much higher than that of the other three size-classes (tests of equality of variances, $p < 0.006$). The model for each size-class of metalimnetic zooplankton is the same as the general ANCOVA model. The factor Boat explains from 32.34% of the variance for size-class 100–202 μm to 39.97% for size-class 202–500 μm (Table 5); neither the total variances nor the sampling design variances differ significantly among size-classes. The analyses show that more than 37% of the total measured variation in the epi- and metalimnion is explained by the sampling design. The differences between the total variances and between the sampling design variances of the two strata are not significant.

Vertical spatial structure

The hypothesis $H(0.1)$ of no significant effect of the sampling design on the spatial structure of the zooplankton size-class data was not rejected for the vertical axis, so the null hypothesis $H(0.3)$ corresponding to hypothesis $H(1.3)$ that a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton was tested using the method of partition of ecological variation of Borcard et al. (1992) (see below).

Impact of the sampling design on the analysis of the zooplankton spatial structure

Hypothesis $H(0.1)$ was rejected for the epi- and metalimnion; the zooplankton data variation was then analysed after controlling for the effect of the sampling design variables. Hypothesis $H(0.2)$ corresponding to hypothesis $H(1.2)$ that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton was tested for both strata using method 1, which combines the ANCOVA, stacked univariate F statistic, and the variation partitioning method (Table 6).

The small differences between the results obtained from methods 1 and 2 for the epi- and metalimnion are caused by the stacked univariate F statistic. It does not take into account the covariances between the size-classes of zooplankton, whereas RDA and partial RDA used in method 2 take these covariances into account. However, the spatial and environmental variables selected by the two methods are the same as well as the fractions (T + TE + TS + TSE), (E), (S), (SE), and (Un) and their probabilities.

In the epi- and metalimnion, the variation after controlling for the sampling design effect (i.e., elimination of fraction (T + TE + TS + TSE)) is explained mainly by the spatially unstructured environmental fraction (E), indicating that the influence of these variables remains the same independently of the positions of the sites. Whereas the unexplained spatial variation, fraction (S), is not significant in the epilimnion and is slight in the metalimnion, a small significant fraction of variation is explained by the spatially structured component of the environmental variables, fraction (SE) (Fig. 4). Therefore, in both strata, the spatial component of the zooplankton variation is slight (epilimnion: (S + SE) = 4.40%, $p = 0.03$; metalimnion: (S + SE) = 6.10%, $p = 0.012$).

If we consider the partitioning of (T + TE + TS + TSE) into fractions (T), (TE), (TS), and (TSE) obtained by method 2, we observe that the partitioning of this fraction is not the same in the two strata (Table 6). In the epilimnion, fraction (T) explains 33.39% ($p = 0.001$) of the total variation, whereas this fraction is 0.42% and not significant in the metalimnion; therefore, the variation generated exclusively by the sampling design (i.e., the factors Day, Boat, and Hour and theirs interactions) is important in the upper layer and nonexistent in the other strata. However, fraction (TSE) is high in the metalimnion (25.25%) as well as fraction (TE), which explains 11.51% of the total variation; this means that

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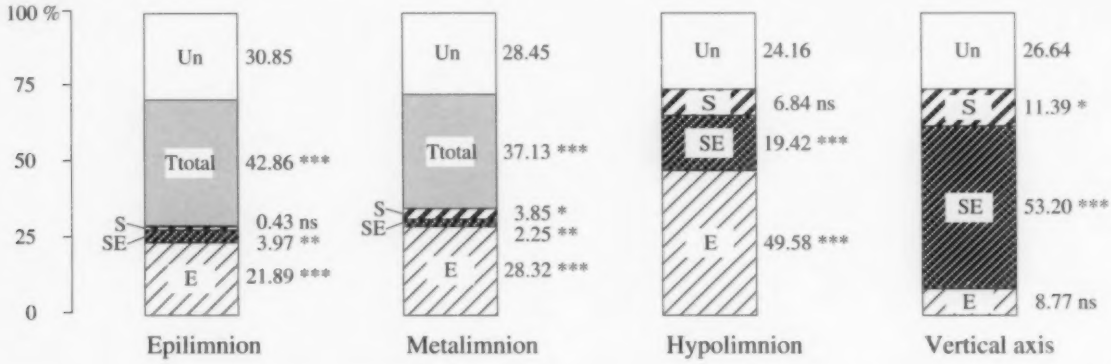
Fig. 4. Partitioning of the variation in the epi-, meta-, and hypolimnion and along the vertical axis. Ttotal represents all components of the sampling design, i.e., (T + TE + TS + TSE). Significant fractions are coded as follows: ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; ns, not significant.



part of the observed environmental variation explained by the environmental variables is influenced by the factor Boat, which dominates the ANCOVA in this stratum (Table 3). These fractions are small in the epilimnion, even negative in the case of (TSE); fraction (TS) explains 12.24% of the total variation, meaning that part of the spatial heterogeneity of the zooplankton is explained by the sampling design factors. Fractions (TS) in the metalimnion and (TSE) in the epilimnion, which are obtained by subtraction (see above) and not by estimating an explicit parameter, are negative. A negative fraction, e.g., (TS) in the metalimnion, indicates that the spatial and sampling design variables, taken together, explain the response variables (i.e., the four size-classes of zooplankton) better than the sum of the individual effects of these factors. This is due to the fact that one or the other (or both) of these groups of explanatory variables has a positive as well as a negative effect on the response variables, one of them being direct and the other indirect (Legendre and Legendre 1998).

These results show that the sampling design generated a high amount of variation in the epilimnion zooplankton, the other important part of the variation being explained exclusively by the environmental variables. In the metalimnion, the measured variation was largely environmental, but the factor Boat generated some environmental and spatially structured environmental variation.

Spatial structure of the zooplankton size-class data

The results obtained from method 1 were verified using method 2; both sets of results are presented in Table 6. For the horizontal and vertical axes, the total variation explained by each model is higher than 69% and is always highly significant ($p < 0.001$).

Horizontal spatial structure

Tests of equality of variances showed that the "total variances" of the three horizontal layers are not significantly different. The "ecological variance" (i.e., fractions (E) and (SE)) was, however, smaller in the epilimnion (mean square (MS) = 0.0670) than in the other analyses (metalimnion: MS = 0.1512, comparison with the epilimnion: $p < 0.01$; hypolimnion: MS = 0.2149, comparison with the epilimnion: $p < 0.01$; results not shown in the tables). However, the difference between the meta- and hypolimnion is not significant.

The spatial heterogeneity of the zooplankton (i.e., fraction (S + SE)) is slight in the horizontal strata, except in the hypolimnion (Fig. 4). The epilimnetic spatial variance (fraction (S + SE): MS = 0.0113 for the epilimnion) is smaller than in the metalimnion (MS = 0.0268, comparison with the epilimnion: $p < 0.01$) and hypolimnion (MS = 0.0744, comparison with the epilimnion: $p < 0.001$), whereas the meta- and hypolimnion show no significant difference. The "pure" spatial variation is small and not significant in the epilimnion and small and marginally significant in the metalimnion; this may indicate that the processes that generate spatial heterogeneity are well captured by the other variables included in the analysis for these two strata. The environmental variables (E) provide the main explanation for the zooplankton variation; they explain 49.58% ($p < 0.001$) of the hypolimnetic variation. In the upper strata, the environmental variation (E) is higher than 20% ($p < 0.001$).

Vertical spatial structure

The spatial heterogeneity of the zooplankton is highest (fraction (S + SE) = 64.59%, $p < 0.001$) in the vertical analysis across the three strata (Fig. 4); the dummy variables coding for strata, as well as their interactions with the X and Y spatial coordinates, were included among the spatial variables coding for this analysis. Part of the spatial heterogeneity, brought out by the spatial polynomial of the geographic (X, Y) coordinates, is significantly explained by the environmental variables (fraction (SE) = 53.20%, $p < 0.001$). The pure environmental variation (E) is not significant and the pure spatial variation (S) is 11.39% ($p = 0.049$), indicating that some variables generating spatial heterogeneity have not been included in the analysis. Along the vertical axis, the total variance of size-class 202–500 µm (MS = 0.1521) is significantly higher than that of size-classes >500 µm (MS = 0.0680, comparison with 202–500 µm: $p < 0.01$) and 53–100 µm (MS = 0.0676, comparison with 202–500 µm: $p < 0.01$).

Role of the environmental factors in determining the spatial variation of the zooplankton

Hypotheses $H(0.2)$ and $H(0.3)$, corresponding to hypothe-

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sis $H(1.2)$ that the abiotic factors play a dominant role in determining the horizontal spatial variation of zooplankton, and hypothesis $H(1.3)$ that a combination of abiotic and biotic factors is necessary to explain the vertical spatial variation of zooplankton, have been tested as a byproduct of the variation partitioning (Table 6). The significant spatial and environmental variables selected during forward selection are presented in Table 7, together with the percentage of the ecological variation that they explain. The order of the variables for each deterministic component in the table is that of their selection by the forward-selection procedure, which was done independently in each group of variables (Table 2).

The description of the zooplankton distribution and the detailed interpretation of the environmental variables explaining zooplankton variation will be the subject of another paper.

Were the abiotic factors playing a dominant role in determining the horizontal spatial variation of zooplankton?

The horizontal spatial heterogeneity of the zooplankton is characterised only by the Cartesian coordinate X of the sites; this variable describes the zooplankton distribution along the east–west axis of the lake. The relationships are hyperbolic in the epi- and hypolimnion (X^4 and X^2, respectively) and sinusoidal in the metalimnion (X^3) (Table 7). We know from Table 6, however, that in each stratum, zooplankton variation is explained mainly by the nonspatially structured component (E) of the environmental variables, and we observe that the abiotic variables are those that explain the highest amount of environmental variation: $(\text{Depth})^2$ in the epilimnion (considering maximum depth of the sampling stations as a physical variable), $(\text{Temperature})^2$ in the metalimnion, and $(\text{DSI})^2$ in the hypolimnion. Some biotic variables also influence the structure of the zooplankton: chlorophyll *a* of phytoplankton >20 μm in the epilimnion, Depth and $(\text{Depth})^2$ in the epi- and metalimnion (now considering these variables as indirect indicators of the spatial distribution of fish), and the density of *Chaoborus* larvae in the deepest stratum. The abiotic variables play a predominant role in explaining the spatial variation of the zooplankton, but zooplankton is influenced by a combination of abiotic and biotic variables.

Was a combination of abiotic and biotic necessary to explain the vertical spatial variation of zooplankton?

The vertical spatial heterogeneity of zooplankton is explained by the dummy variables describing the strata and by various multiplicative terms between the Cartesian coordinate X and the hypolimnetic stratum (H) characterising simultaneously the vertical and horizontal axes (Table 7). No biotic variables were selected during the forward-selection procedure. This implies that the biotic factors measured during our study do not significantly explain the vertical heterogeneity of zooplankton. So no biotic variable was accepted in the model. On the other hand, a great part of the zooplankton heterogeneity is explained by the abiotic variables. Therefore, hypothesis $H(0.3)$ that a combination of abiotic and biotic factors is not necessary to explain the vertical spatial variation of zooplankton cannot be rejected. The vertical spatial variation of the zooplankton is influenced only by the vertical gradient in temperature, oxygen, turbidity, pH, and $(\text{DOC})^2$.

Table 7. Sampling design, spatial, and environmental variables contributing significantly to the explanation of the variation measured in the epi-, meta-, and hypolimnion and along the vertical axis.

Deterministic component	Explanatory variable	Explained variation
Epilimnion		
Sampling design	ANCOVA model	0.43***
Spatial	X^4	0.04**
Environmental	$(\text{Depth})^2$	0.10***
	pH	0.05**
	$(\text{Alkalinity})^2$	0.05**
	Chloro. >20 μm	0.03*
	Conductivity	0.03*
Metalimnion		
Sampling design	ANCOVA model	0.37***
Spatial	X^3	0.06*
Environmental	$(\text{Temperature})^2$	0.11**
	Depth	0.05*
	DOC	0.07**
	$(\text{Depth})^2$	0.08**
Hypolimnion		
Spatial	X^2	0.26*
Environmental	$(\text{DSI})^2$	0.45**
	Chaoborus density	0.24***
Vertical axis		
Spatial	Stratum E	0.03***
	$X^4\text{H}$	0.14**
	$X\text{H}$	0.08*
	$X^2\text{H}$	0.07*
Environmental	Oxygen	0.28**
	Turbidity	0.18***
	pH	0.05*
	$(\text{DOC})^2$	0.05*
	Temperature	0.06*

Note: ANCOVA model refers to the general models in Table 5. X^n represents site coordinates to the power n; Chloro. >20 μm, chlorophyll *a* concentration of phytoplankton >20 μm; E, epilimnion; H, hypolimnion. Probabilities were obtained after 999 permutations; significant fractions are coded as follows: $^{***}p \le 0.001$; $^{**}p \le 0.01$; $^{*}p \le 0.05$; ns, not significant. The percent explained variation is the conditional effect, i.e., after considering the other variables selected in the model.

Discussion

Variation generated by the sampling design

The variation generated by the sampling design in the epi- and metalimnion may be related to the number of sites; 50 and 36 sites were sampled in the epi- and metalimnion, respectively, whereas there were only 13 sites in the hypolimnion and along the vertical axis. Since the sites were not sampled simultaneously, increasing the number of sites may have caused temporal variation characterised by the factors Day and Hour. The factor Boat may have generated a further spatial artefact caused by the route of the boats visiting the sampling sites in the given sequence (Ibanez 1973). If this is the case, the factor Boat is more like a spatial sampling de-

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sign factor than temporal, and the greater the number of sampled sites, the greater the spatial artefact should be. Patalas and Salki (1993) indicated that the spatial structure of organisms depended on the number of sites sampled in a lake; indeed, more sites sampled in the same lake involves a more accurate measure of the variability. Other authors add that spatial heterogeneity increases rapidly with the extent of the sampling programme (e.g., Patalas 1990); this supports the idea that the design variation generated during sampling may depend on the number of sites. If one cannot reduce the design variation through the sampling strategy, it must be taken into account during the statistical analysis.

The sampling design variation in the metalimnion is explained only by the factor Boat. This may indicate that this part of the variation is a spatial artefact which can explain the importance of fractions (TE), (TS), and (TSE) and the small importance of the pure sampling design fraction. The Boat effect is confounded with the spatial structure described by the spatial variables (fractions (TS) and (TSE)), and it explains environmental variation that is spatially unstructured (i.e., unexplained by the spatial variables), but it remains correlated with the spatial artefact generated by this factor (TE). Moreover, the factor Boat has a positive as well as a negative effect on the response variables; since the correlation is positive with dummy variables Boat 1 and negative with Boat 2 (result not shown in the tables), this might explain the negative fraction (TS). Taking depth-integrated hauls has eliminated vertical heterogeneity, which can vary over short time periods as a result of vertical migrations (Colebrook 1960); this could explain in part the fact that the metalimnetic zooplankton did not show significant temporal variation, so that fraction (T) is small and not significant. The same sampling method has been used in the epilimnion; however, the total sampling design variation is explained by the interaction terms Hour × Day and Hour × Boat that may characterise not only the spatial artefact but also a temporal variation that could explain the importance of fraction (T) compared with the other fractions. The horizontal temporal variation may be explained by the predation pressure from the fish and (or) the *Chaoborus* larvae and (or) by the source of food. However, only chlorophyll *a* of phytoplankton >20 µm is correlated with the sampling design factors (i.e., interaction term Hour × Boat: $r = 0.594$, $p < 0.0001$). This may explain fraction (TE), but it would indicate, in this case, that the horizontal temporal variation is generated by other variables that have not been included in the study.

Zooplankters are able to accomplish diurnal horizontal migrations (Davies 1985), although they have little directional movements horizontally beyond a few metres, whereas they can move by more than 10 m vertically in response to diel changes in light levels or food. The wind-induced currents may also generate temporal variation; Pinel-Alloul and Pont (1991) indicated a correlation between wind direction and the spatial distribution of macrozooplankton in Lake Cromwell. To affect the spatial heterogeneity of zooplankton, the windward must be regular. The dominant wind was mostly from the southwest during the 10 days prior to sampling, but the day before sampling, the wind was from the northeast (9 $km \cdot h^{-1}$). During the sampling days, it turned southwest again. This could explain the greater concentration of zooplankton in the central part of the lake when the wind was from the north-northeast. Together with the swimming capabilities of the species (Steele 1978), the wind direction change may explain the temporal variation in the epilimnion (i.e., fraction (T)) as well as fraction (TS), which characterised the spatiotemporal variation of the zooplankton. However, since Lake Cromwell is small and sheltered by forest, the changes in wind direction possibly had negligible effects on the distribution of zooplankton. In that case, the sampling design variation would be a complete artefact, since the sampling sites were not the same during the two days and the number of sites sampled in the epilimnion was higher than in the metalimnion. This may have generated a temporal artefact, which is not found in the metalimnion because of the smaller number of sites visited. So the effect of the factor Boat would increase more rapidly with the number of sites sampled than the effects of the factors Day and (or) Hour.

Some studies on the distribution of zooplankton have been carried out at broad temporal and spatial scales (e.g., Gaudy et al. 1995; Masson and Pinel-Alloul 1998). However, in many studies on the spatial structure of organisms where sampling was carried out over a few hours or days, spatial variation analysis has not taken the temporal effect into account because daily and short-term variations were considered negligible (e.g., Gaudy et al. 1995; Lacroix and Lescher-Moutoué 1995). Previous studies in Lake Cromwell showed that the sampling date was not a significant predictor of the spatial heterogeneity of zooplankton, suggesting no short-term effect. On the contrary, the spatial heterogeneity along the vertical axis was found to be related to the hour of sampling (Pinel-Alloul and Pont 1991). However, none of these studies took into account the route of the boat or the interaction that could exist between the factors of the sampling design, and the sampling did not cover the whole lake, since it was carried out in a delimited rectangular area of the lake or along a transect (e.g., Pinel-Alloul and Pont 1991).

Impact of the sampling design on the detected zooplankton spatial structure

If we compare the analyses without and with control for the sampling design effect (Fig. 5), we find that the partition of the measured variation is not the same in the two analyses; nor are the same explanatory variables selected by the forward-selection procedure.

Epilimnetic stratum

If we had studied the epilimnetic zooplankton variation without controlling for the sampling design, we would have concluded that 75.25% of the measured variation remains unexplained by the environmental and spatial variables, which would indicate that some important processes generating ecological variation have not been included in the study. However, considering the effect of the sampling design, 69.15% of the measured variation is explained by the variables selected. The decrease in unexplained variation demonstrates the importance of the variation generated exclusively by the sampling design (i.e., fraction (T)), showing that fraction (S + SE + E), which represents the variation explained by the spatial and environmental variables, does not change in any drastic way (fraction (S + SE + E) = 24.75 and 26.29%, without and after controlling for the sampling

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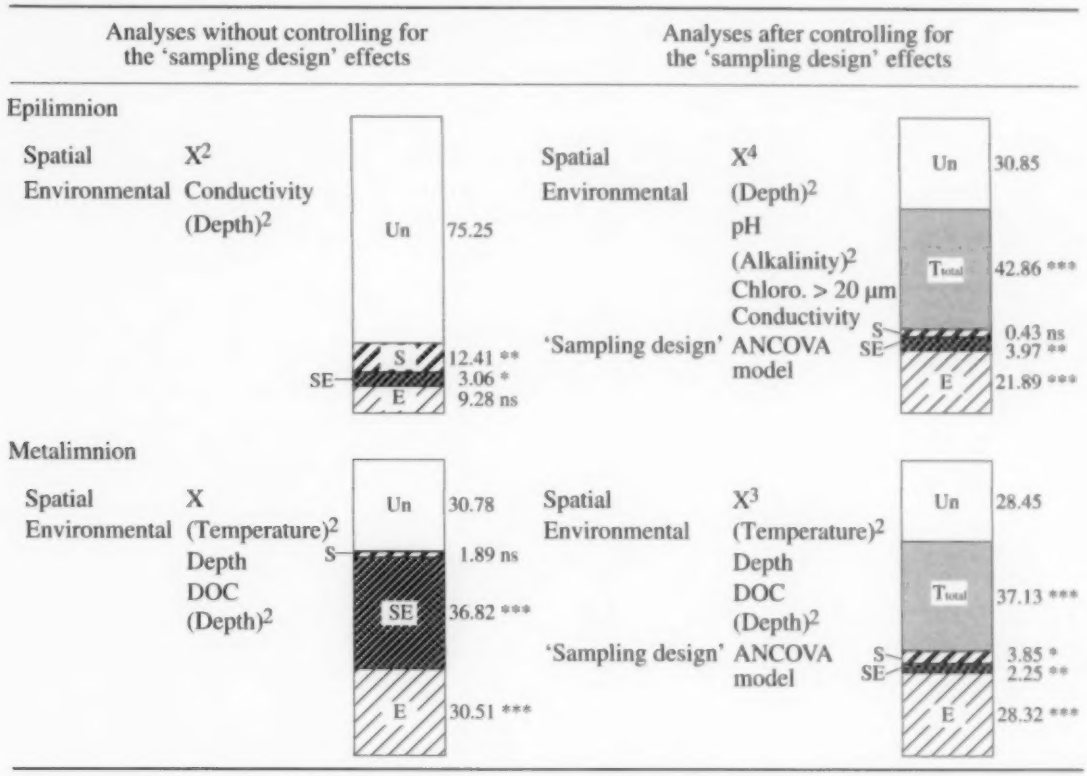
Fig. 5. Sampling design affecting the analysis of the zooplankton variation. Sampling design, spatial, and environmental factors are the significant variables selected in the analyses. The measured variation represented by the fractions is expressed as a percentage. X^a represents site coordinates to the power a; Chloro. >20 μm, chlorophyll a concentration of phytoplankton >20 μm. Significant fractions are coded as follows: ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; ns, not significant.



design, respectively). This is not the case for the metalimnion (see below).

The pure environmental variation characterising the functional structure of the zooplankton (E) in the epilimnion, which is not significant in the analysis that did not control for the spatial design, now represents 21.89% of the total variation and is significant. The spatial structure of the zooplankton remains unchanged (significant spatial factor X^2 in the first analysis and X^4 in the second) but the environmental variables are not the same; without controlling for the sampling design, conductivity is the main variable explaining the measured variation. In the second model, on the contrary, the environmental variables corroborate most of the conclusions of the previous studies in Lake Cromwell (e.g., Pinel-Alloul and Pont 1991, see below).

Metalimnetic stratum

Contrary to the epilimnion, the total explained variance remains largely unchanged in the metalimnion, without or after controlling for the sampling design (69.22 and 71.55%, respectively). The pure spatial variation (S) as well as the unexplained (Un) and spatially unstructured (E) environmental variations are unchanged in the two analyses, whereas the ecological spatial variation ((SE): 2.25%, $p < 0.01$) is less important than in the analysis without controlling for the sampling design (36.82%, $p < 0.01$). It corroborates the spatial artefact caused by the factor Boat, which is confounded by the spatial structure of the zooplankton and the nonexistence of a pure sampling design variation (i.e., fraction (T)).

In both strata, controlling for the sampling design decreased the estimated amount of the variation which was attributed to the spatial structure of the zooplankton (SE + S), although the effects of the sampling design remain different in the two strata.

Variation of the zooplankton size-class data

Horizontal variation

In the horizontal strata, there are no significant differences in total variation (after controlling for the sampling design) among the four size-classes of zooplankton, except for size-class 202–500 μm (MS = 0.0638) in the epilimnion, which shows a total variation significantly higher than that of size-classes 100–202 μm (MS = 0.0282, comparison with 202–500 μm: $p < 0.01$) and 53–100 μm (MS = 0.0211, comparison with 202–500 μm: $p < 0.001$). The heterogeneity among size-classes of zooplankton for the three strata (using stacked univariate F statistics) differs in a significant way for size-class 202–500 μm (MS = 0.0687); its variation is higher than that of size-classes >500 μm (MS = 0.0409, comparison

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with 202–500 μm: $p < 0.01$) and 53–100 μm (MS = 0.0407, comparison with 202–500 μm: $p < 0.01$). The differences could be related to the trophic requirements and swimming capabilities of the species (Steele 1978) and to their patterns of vertical migration (Pinel-Alloul and Pont 1991). Body size has also been considered a specific component of plankton heterogeneity in Lake Cromwell when all zooplankton groups, from small rotifers to large copepods, are included in the analysis (Pinel-Alloul et al. 1988). This could also explain the importance of the sampling design variation of size-class 202–500 μm in the epilimnion, which is significantly higher than that of the other size-classes and would suggest that part of the sampling design variation is temporal variation inherent to this size-class rather than an artefact caused by the sampling.

Otherwise, how can one explain the fact that the sampling design effect on this size-class is greater than on the others size-classes? Adaptation specifically associated with horizontal spatial variation may be a major feature in some zooplankton species but not in others (Lewis 1978). Pinel-Alloul and Pont (1991) indicated that the heterogeneity among the macrozooplankton species of Lake Cromwell (which corresponded to sizes 430–1430 μm in their study) showed no significant differences on the horizontal axes and with depth over the horizontal axes, whereas a strong species effect was noted along the vertical axis. The differences between the two studies could be due to the range of body sizes of the organisms. In the same study, the authors pointed out that the maximum spatial heterogeneity in relation to population abundanceswas observed along the vertical and not the horizontal axis. However, a stronger aggregation was also observed in the hypolimnion for the total zooplankton whose spatial heterogeneity increased with depth (Pinel-Alloul et al. 1988). Surface turbulence and advective processes in surface waters may have had a randomising effect, whereas thermal stratification may have induced high spatial heterogeneity (Pinel-Alloul and Pont 1991). Moreover, the predation pressure by *Chaoborus* larvae may be higher in deeper waters, yielding aggregation as a predation-avoidance behaviour (e.g., Masson and Pinel-Alloul 1998).

The abiotic variables play an important role in the horizontal spatial variation of zooplankton, except in the epilimnion where the spatial distribution of the zooplankton is also influenced by phytoplankton >20 μm (e.g., Richerson et al. 1978). The ecological variation along the horizontal axes is generated by a combination of the abiotic and biotic variables. Planktivorous fish (*Notropis cornutus*, *Lepomis gibbosus*, and *Exoglossum maxillingua*) occur in greatest concentration in the littoral zone of Lake Cromwell (Pinel-Alloul and Pont 1991). Fish echo-location data obtained by echosounder in Lake Cromwell during the summer of 1994 showed a spatial distribution of fish from the littoral to the pelagic zone with low abundance of fish in the pelagic zone and high abundance in the littoral zone (Gaudreau and Boisclair 1998). The presence of planktivorous fish would explain the smaller spatial heterogeneity in the epilimnion, since the predation pressure seems to be an essential variable in the maintenance of horizontal patterns in zooplankton distributions (e.g., Gliwicz and Rybowska 1992). Two species of *Chaoborus* are present in Lake Cromwell: *Chaoborus flavicans* is more or less restricted to the littoral zone, whereas *Chaoborus punctipennis* inhabits the pelagic zone, where its highest density is 3374 individuals·m^{-2} (Harper and Cloutier 1986). The *Chaoborus* larvae move deeper with age (Fedorenko and Swift 1972); that may explain the influence of *Chaoborus* larvae density on the zooplankton community in the hypolimnion.

Vertical variation

The vertical heterogeneity of the zooplankton data is explained exclusively by the abiotic variables and not by a combination of the abiotic and biotic variables, as was assumed by our hypothesis $H(1.3)$. All sites analysed along the vertical axis are pelagic and their number is small (13 sites) due to the morphology of the lake. The 26.64% unexplained vertical variation indicates that others biotic factors that were not considered during the study could explained the vertical heterogeneity of zooplankton. However, the environmental variables inducing environmental and spatial heterogeneity of the zooplankton are in agreement with the results of previous studies conducted in Lake Cromwell (Pinel-Alloul et al. 1988; Pinel-Alloul and Pont 1991). Our results support the idea that physical stratification is at the origin of the biological heterogeneity among strata (Richerson et al. 1978).

In conclusion, in ecological field studies, visiting prelocated sites in some random order (Ibanez 1973) or sampling all sites simultaneously are certainly the best sampling strategies, but they are utterly unrealistic for field studies. Any other sampling strategy is not random and introduces dependencies among the observations, which must be taken into account during the following phases of analysis and interpretation of the data. The magnitude of this effect depends, however, on the generation time or the ability of the organisms to move relative to the duration of the sampling campaign.

Most sampling designs through geographic space cannot guarantee a correct estimation of the spatial variation of a set of variables. We must always ask what is the real nature of the variation measured using our sampling design. The design may modify in a significant way the study of the spatial variation of a community and of the contribution of the environmental variables sampled concomitantly. The first step is to take into account a possible effect of the sampling design and thus not consider a priori the daily or short-term temporal variation as well as the route of the boats as negligible artefacts without testing this hypothesis. If a significant effect of the sampling design is found, one must control for it during the following analysis and interpretation of ecological variation.

If the effect of the sampling design is identified during a pilot study (Amanieu et al. 1989; Fortin et al. 1989), an alternative solution is to change the sampling strategy before the full-size study is undertaken. The measured variation depends, however, on the number of sites sampled as well as the duration of the sampling programme. In some research, a large sampling area may be required, so we may not want to limit the sites visited to some small number.

The traditional methods of sampling (Wisconsin plankton net, Clarke–Bumpus sampler, Schindler–Patalas trap) do not permit the filtration of large volumes of water and require an appreciable amount of time to sample a site. In the last two

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decades, four advanced technologies have been developed: acoustic devices, optical plankton counters, video systems, and laser fluorosensors. Although they have limitations, these sampling methods would permit researchers to rapidly cover a large lake surface area (for reviews, see Williamson et al. 1992; Pinel-Alloul 1995). Besides these high-technology methods, motorised pumps seem to be the best sampling gear for volumetric sampling. They permit the filtration of large, controlled volumes of water rapidly and can be manipulated easily in order to obtain integrated water samples covering the scale of a lake (e.g., Rahkola et al. 1994; Lacroix and Lescher-Moutoué 1995).

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DISCUSSION

Comment: Larval Atlantic cod and haddock growth models, metabolism, ingestion, and temperature effects[1]

Lawrence J. Buckley, R. Gregory Lough, Myron A. Peck, and Francisco E. Werner

In a recent article, Leising and Franks (1999) presented detailed individual-based models (IBMs) for larval Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) based on the bioenergetic models of Laurence (1985) for the same species. Using these models, they drew conclusions on the effects of varying prey density, turbulence, and temperature on larval growth rate. However, an error in calculation of metabolism and their choice of a scalar for the effect of temperature on assimilation (Q_a, their table 1) may require a revision of Leising and Franks' (1999) conclusions. The error in estimation of daily metabolic rates originated in Laurence (1985) and was repeated in Werner et al. (1996). In this comment, we provide revisions for the estimation of metabolic costs and suggest that Q_a for marine fish larvae should fall in the range of 1.06–1.14 based on our analysis of available literature. We also point to components of the model where compensating errors may occur and additional research is needed.

Revision of estimates of metabolic costs

The seminal modeling work of Laurence (1977, 1985) and Beyer and Laurence (1979, 1980, 1981) on marine fish larvae has provided much of the groundwork for later IBMs for fish larvae. However, it appears that assumptions, parameter estimates, and even errors have not been critically examined in the intervening years. Consequently, they continue to propagate through the modeling literature. The formulation used by Leising and Franks (1999) and Werner et al. (1996) for daily metabolic rates is a case in point.

The method employed by Laurence (1985) to convert from routine hourly rates to total daily metabolism was outlined in Laurence (1977) and Beyer and Laurence (1980). Hourly metabolic rates reported in Laurence (1975, 1978) were considered to be "routine" after the terminology of Fry (1971), since larvae were able to make routine movements within the respirometer chambers. These routine rates were thought to apply during daily resting periods and at night (Beyer and Laurence 1979). Larval metabolism was a function of weight (W) of the form MW^b, and the assumption was made that the weight exponent (b) was constant and only the k value changed with activity level. Laurence (1977) and Beyer and Laurence (1980) used a k ratio (active/routine metabolic rate) of 2.5 to convert from routine to active rates. This value was taken from the work of Ware (1975) who reported a range of values from 2 to 3 in adult fish. Beyer and Laurence (1979) considered in detail the metabolic costs due to larval activity (e.g., searching and attacking prey) and derived a k ratio of 1.9 for herring larvae using swimming speeds of 0.5 and 1 body length·s^{-1} for routine and active rates, respectively.

The hourly rates of oxygen consumption (y, microlitres of oxygen per larva per hour) reported by Laurence (1978) for larvae at 7°C were

$$y = 0.017W^{0.775} \text{ (Atlantic cod)}$$

$$y = 0.071W^{0.684} \text{ (haddock)}.$$

where W is the dry weight in micrograms.

The daily metabolic rates (M, microlitres of oxygen per larva per day) reported in Laurence (1985) for larvae at 7°C were

$$M = 24(0.010W^{0.775}) \text{ (Atlantic cod)}$$

$$M = 24(0.038W^{0.684}) \text{ (haddock)}.$$

However, these estimates of daily metabolism were not consistent with the outlined method for converting from routine to active rates.

With light regimes of 13 h light : 11 h dark for Atlantic cod and 14 h light : 10 h dark for haddock and by using a

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conversion factor (k ratio) of 2.5, the daily metabolic rates should be

(1) $M = 43.5(0.017W^{0.775})$ or $0.74W^{0.775}$ (Atlantic cod)

(2) $M = 45.0(0.071W^{0.684})$ or $3.20W^{0.684}$ (haddock)

if the procedure outlined in Laurence (1977) and Beyer and Laurence (1980) is followed (e.g., for Atlantic cod (13 × 2.5) + 11 = 43.5). Although Laurence (1985) did not explicitly state the k ratio used, we can assume that the value should be close to 2. It appears that Laurence (1985) may have divided the routine rate by 2.5 rather than multiplied (e.g., for haddock: 0.071 × 24/45 = 0.038), since the daily rates are less than 24 times the reported routine hourly rates. Our recalculated estimates of daily oxygen consumption above are roughly three times higher than those reported in Laurence (1985). The lower and presumably erroneous estimates of daily metabolic rates have been incorporated into most bioenergetic models of Atlantic cod and haddock published since 1985, even when time steps shorter than 1 day were used (Werner et al. 1996; Leising and Franks 1999).

Since metabolic rates (energy losses) were apparently underestimated in Laurence (1985), Werner et al. (1996), and Leising and Franks (1999) by a factor of 2–3 and growth rates were within the expected range, compensating errors may be present in other components of the models. Two possibilities are that (*i*) the routine hourly metabolic rates of Laurence (1978) or the k ratio may have been overestimated or (*ii*) the rates of food consumption or assimilation (energy gained from feeding) may have been underestimated. We expand on these points next.

Metabolic rates

More than 20 years ago, Beyer and Laurence (1979, p. 28) stated "Development of metabolic models that are other than tentative is difficult. The basic problem is that we lack simultaneous quantitative measurements of activity and oxygen consumption of larval fish...." This statement remains valid today. The most relevant data are those of Hunt von Herbing and Boutilier (1996) for Atlantic cod larvae reared at 5°C and tested at 10°C in a respirometer at speeds ranging from 0.2 to 1.0 mm·s^{-1}. Although the maximum swimming speed reported in Hunt von Herbing and Boutilier (1996) was less than the 1 body length·s^{-1} value used by Beyer and Laurence (1979) for active metabolism, a doubling of swimming speed yields a k ratio of about 1.6 for Atlantic cod larvae. This estimate is close to the value of 1.9 for herring larvae derived from a detailed consideration of the metabolic costs of activity and feeding (Beyer and Laurence 1979).

Assimilation

Laurence (1985), Werner et al. (1996), and Leising and Franks (1999) followed Anderson and Ursin (1977) and reduced the ingested food (I) available for growth by incorporating the terms $(1 - \alpha)$ and β into the growth equation

$$G = [(1 - \alpha)\beta I] - M$$

where α (set at 0.4) is the specific dynamic action (SDA), the cost of digesting and processing food, and β is the assimilation efficiency, the proportion of ingested food not lost as excreta. In the Laurence (1985), Werner et al. (1996), and Leising and Franks (1999) models, β was a function of larval size, increasing from 0.3 to 0.8 during development. This estimate of β was based on work with a single species, summer flounder (*Paralichthys dentatus*) (Buckley and Dillman 1982). Together under this formulation, the α and β terms (representing SDA and egestion) reduced the total ingested energy available for growth by 50–80%, with the smallest larvae realizing the least benefit from ingested food. More recent data indicate that fish larvae may be more efficient than previously thought at converting ingested matter into growth (Kiørboe et al. 1987; Wieser and Medgyesy 1990; Letcher et al. 1996). The very low assimilation efficiencies of young larvae may be underestimated. Letcher et al. (1996) used 0.6 as the minimum value of β, rapidly rising to 0.8 with increases in fish size. Current thinking on SDA suggests that it mainly represents the cost of biosynthesis, primarily synthesis of protein, and that SDA accounts for only about 10% of the energy available for growth or about 14% of ingestion (Kiørboe et al. 1987). Additional research is needed on the effects of development and temperature on assimilation and growth efficiency during the larval period.

Revision of temperature dependence

In Leising and Franks (1999), larval growth rates and consequently conclusions on the effects of temperature on growth were strongly dependent on the scalar, Q_a, used to adjust assimilation for the effect of temperature. In their model, assimilation (A) was temperature dependent:

(3) $A = Q_a^{(T-T_{ref})}\beta I_{ref}$ or $I_T = Q_a^{(T-T_{ref})}I_{ref}$

where T_{ref} is the reference temperature (set at 7°C for both Atlantic cod and haddock), I_{ref} is the rate of ingestion (micrograms) at the reference temperature, and I_T is the ingestion at temperature T. The value of Q_a used (1.6 for both Atlantic cod and haddock) was not based on empirical data but chosen so that the model reproduced available growth data. Their estimate of Q_a is high. A Q_a of 1.6 results in a 60% increase in assimilation for a 1°C rise (156% for a 2°C rise) from the reference temperature or a Q_{10} of 110. Since assimilation efficiency (β) in fish larvae appears to decrease with increasing temperature (Houde and Zastrow 1993), any increase in assimilation with temperature must be a result of increased ingestion.

The use of the scalar Q_a to adjust ingestion for temperature is based on the assumption that ingestion increases exponentially with temperature. This may or may not be the case, since, as pointed out by Leising and Franks (1999), "With no data concerning the functional responses of these factors to temperature...other formulations for temperature dependence may be equally valid." Although there is very little in the published literature on fish larvae to determine the shape of the relationship between ingestion and temperature, for small changes in temperature the value of the temperature scalar may be more important than the shape of the relationship. The use of a scalar to determine food consumption at different temperatures represents a compromise forced by a lack of information. Ingestion in most IBMs is driven by encounters between predators and prey, estimated from swimming speeds and turbulence, among other factors. Running these models at different temperatures requires ad-

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ditional data on metabolism, activity, and swimming speed of both predator and prey, as a function of temperature, as well as data on appetite, capture success, and digestion rates at different temperatures.

Based on a synthesis of data for larvae of a wide variety of species, Houde and Zastrow (1993) reported a linear relationship between weight-specific ingestion per day (I_A) and water temperature:

$$I_A = 0.0144 + 0.0338T$$

where T is water temperature (degrees Celsius). Multiplying both sides of this equation by dry weight (W, micrograms) yields ingestion (I, micrograms per day). Based on this relationship, ingestion at any temperature (I_T) can be calculated from ingestion at a reference temperature (I_{ref}) using the equation

$$(4) \quad I_T = I_{ref} + I_{ref}(T - T_{ref})/(0.426 + T_{ref}) = I_{ref}(1 + c(T - T_{ref}))$$

where I_T and I_{ref} are in micrograms per day and $c = 1/(0.426 + T_{ref})$. This equation becomes

$$I_T = I_7(1 + 0.135(T - 7))$$

when T_{ref} is set to 7°C. In this case, ingestion increased linearly with temperature with the slope dependent on the reference temperature. For small changes in temperature about $T = T_{ref}$, eq. 3 can be linearized to $I_T = I_{ref}(1 + (\ln Q_a)(T - T_{ref}))$. So by comparison with eq. 4, $c = \ln Q_a$ or $Q_a = \exp(1/(0.426 + T_{ref}))$, which gives $Q_a = 1.14$ for $T_{ref} = 7$ and $Q_a = 1.07$ for $T_{ref} = 14$.

A Q_a value also can be estimated from model 5 in MacKenzie et al. (1990):

$$\log_{10} I = 1.162 \log_{10} W + 0.029T - 1.343$$

derived from a synthesis of data for a variety of species. Based on this relationship, ingestion at any temperature can be calculated from ingestion at a reference temperature using

$$\log_{10} I_T = \log_{10} I_{ref} + 0.029(T - T_{ref})I_T$$

or

$$I_T = I_7 \times 1.069^{(T - T_{ref})}$$

indicating that Q_a is equal to 1.069 and is independent of either larval weight or T_{ref}.

Letcher and Bengtson (1993) published equations for ingestion by young inland silverside (*Menidia beryllina*) as a function of weight at three temperatures. Based on these equations for fish fed at maximum rations, we calculated Q_a values for larvae of different sizes. The Q_a for inland silverside was dependent on weight according to the equation

$$Q_a = 0.039W \times 10^{-3} + 1.066$$

indicating that Q_a increased from about 1.07 to 1.14 during the larval period (100–2000 µg dry weight).

Our analysis of the published data on fish larvae (MacKenzie et al. 1990; Houde and Zastrow 1993; Letcher and Bengtson 1993) indicated that Q_a falls within the range of 1.06–1.14 (Q_{10} of 1.8–3.7) and may be dependent on larval size, prey availability, and temperature. If Leising and Franks (1999) had used an estimate for the physiological rate quotient within this range, then their Q_a would have been much closer to their choice of a value for the respiratory quotient ($Q_r = 1.07$). Consequently, their modeled effects of temperature on growth would have been subtler and presumably more realistic. Since in their model, it is the ratio of Q_a to Q_r that in large part determines the magnitude and sign of the change in growth with temperature, their choice of a high Q_a biased the model outputs toward rapidly increasing growth with increasing temperature. The conclusion of Leising and Franks (1999) that "Increasing the average temperature cycle by 1°C...increased the growth of larvae that were not growing well previously" should be reexamined. This conclusion also runs counter to field observations of a temperature optimum for growth (T_{opt}) of Atlantic cod and haddock larvae between 6 and 7°C (Campana and Hurley 1989; L.J. Buckley et al., unpublished data). The occurrence of an optimum temperature for growth suggests that at ambient prey levels in the field, any increase in ingestion or assimilation with temperature above T_{opt} was insufficient to meet the increased metabolic costs at higher temperatures.

To summarize, in this contribution, we provide corrections (eqs. 1 and 2) to the estimates of daily metabolism in Laurence (1985). The increase in the daily metabolic costs should decrease modeled growth and therefore suggests that compensating errors may occur elsewhere in the models. We also show that the Q_a value used by Leising and Franks (1999) (to adjust assimilation for the effect of temperature) is high in comparison with available information. Based on an analysis of relevant literature, we suggest that Q_a for marine fish larvae should fall between 1.06 and 1.14. Use of a smaller Q_a value within the suggested range will greatly reduce the rate of change in larval growth with temperature, most likely reducing growth estimates at temperatures above T_{ref} and increasing growth estimates below *T*ref. Since these questions affect key elements of the analysis performed by Leising and Franks (1999), their conclusions may need to be revisited, particularly with respect to temperature. Significant improvements to larval Atlantic cod and haddock growth models will require additional research on swimming and feeding behaviors, active metabolism, and assimilation efficiency as well as the effects of development, temperature, and food availability on these processes.

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Reply to Buckley et al.'s "Comment: Larval Atlantic cod and haddock growth models, metabolism, ingestion, and temperature effects"[1]

Andrew W. Leising and Peter J.S. Franks

Introduction

In reexamining recent modeling work on Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae, Buckley et al.[1] conclude that the procedure used in Laurence (1985) to calculate daily metabolic rates may have been incorrect. Buckley et al. suggest that the actual daily metabolic rates should be nearly three times higher for both Atlantic cod and haddock. At the same time, they also point out that total assimilation may be much higher (70% rather than <50%) based on more recent data, including laboratory experiments conducted on larval fish of other species (Kiørboe et al. 1987; Wieser and Medgyesy 1990; Letcher et al. 1996).

Besides these points, Buckley et al. suggest that the value of Q_a, a temperature-dependent "overall" assimilation term used by Leising and Franks (1999), was unrealistically high (we used a value of 1.6). Instead, Buckley et al. suggest that this value should be closer to 1.07–1.14 based on their analysis of data from several experiments on other species of fish. At the time of creation of our model (Leising and Franks 1999), we used our best estimates of these parameters. However, in light of Buckley et al.'s arguments, we agree that their estimates for these parameters may be more appropriate for Atlantic cod and haddock larvae. We therefore reran our model for Atlantic cod only using their suggested parameters to determine the impact on our model results, with specific attention to the effects of temperature versus food availability.

Model forcing

We raised the value of *mc*, the coefficient of respiration, according to Buckley et al.'s recalculation, from 0.01 to 0.031 μL O_2·larva^{-1}·h^{-1}. We also put a limit on the effects of *a* and gamma (assimilation efficiency and specific dynamic action, respectively) so that at most, total assimilation would not be reduced by more than 30%, as suggested. We then set the value of Q_a to their higher suggested value of 1.14. The model from Leising and Franks (1999) was then rerun using these new parameters, with all other parameters the same as before. Under this new parameter set, however, growth was unrealistically high at the reference prey concentration of 15 μg C·L^{-1} and the reference temperature of 7°C (at the reference temperature, there are no effects of the temperature-dependent portions of our model versus previous non-temperature-sensitive models). Under these settings, average dry weight after 40 days was 11 500 μg, compared with the target weight of 2500 μg (this target weight was based on weight-at-age measurements from field-sampled larval Atlantic cod, where age was determined by otolith measurements (Bolz and Lough 1988)). This unrealistic growth resulted because even though the metabolic rate was three times higher here than in Leising and Franks (1999), it does not properly balance the increase in assimilation efficiency (from our previous value of <50% to the suggested value of >70%).

In order to make the model results more realistic compared with field and laboratory measurements of growth, we therefore lowered the maximum allowed growth rate per day from 15 to 11%. Whereas the previously used maximum growth rate of 15%·day^{-1} was based on extrapolations from laboratory measurements (reported in Laurence 1985), measurements of otoliths from field-collected larvae (Bolz and Lough 1988) suggest that maximum daily growth rates fall in a range from 11.4 to 13.7% over the first 40 days of life for Atlantic cod larvae. Therefore, we felt that reducing our maximum growth rate to 11% in order to balance the suggested increase in assimilation efficiency was not unrealistic.

With the lower adjusted value of maximum growth rate, the model was then run at 5, 7, and 9°C with either 5, 7, 10, 15, or 25 μg prey C·L^{-1} available as food and with a Q_a of 1.14. Each model run was for 40 days, as in Leising and Franks (1999), but with only 50 individual larvae. Average larval weight (and standard deviation) (micrograms dry weight) after the 40 days was calculated (Fig. 1).

Results

Using the new parameter set, average dry weight (and standard deviation) after 40 days was similar to our previous result at 7°C (noting that we fit the model to match the previous result by altering the maximum daily growth rate). At this temperature and with the new settings, average growth increased as food concentration increased, up to a prey concentration of 10 μg C·L^{-1} (Fig. 1). Below a prey concentra-

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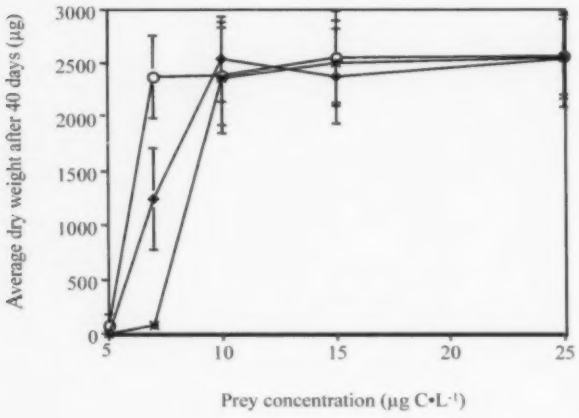
Fig. 1. Average dry weight of Atlantic cod larvae after 40 days (n = 50 larvae for each run) versus concentration of prey and at three different temperatures (asterisks, 5°C; diamonds, 7°C; circles, 9°C) using the new parameter values suggested by Buckley et al. to force the temperature-dependent portions of the model. Error bars represent ±1 SD.



tion of 7 µg C·L^{-1}, larval weights were low enough that they probably would not survive much beyond the 40 days simulated here. At 9°C, the average dry weight after 40 days reached its maximum at all prey concentrations of 7 µg C·L^{-1} and above (Fig. 1). Average dry weight rapidly decreased below a prey concentration of 7 µg C·L^{-1}. At 5°C, average dry weight did not reach its maximum until prey concentrations reached at least 10 µg C·L^{-1} (Fig. 1).

Discussion

Under the new parameter set, it appears that the larval Atlantic cod are food limited at prey concentrations of 7 µg C·L^{-1} or less and limited by their maximum allowed growth rate at concentrations of 10 µg C·L^{-1} and higher. Temperature, in these cases, appears to set the threshold prey concentration at which the larvae switch between these two extremes. Unlike the results of our previous model parameterization, the current model has this switch occurring over a much narrower range of prey concentrations (compare with fig. 5 in Leising and Franks 1999). Altering the maximum allowed growth rate or the minimum assimilation efficiency parameters would alter the range over which this threshold occurs. However, the main conclusion concerning temperature effects remains unchanged. This conclusion — increasing temperature can increase the growth rates of the larvae when they are food limited — is the same as our conclusion under our previous parameter set. For real Atlantic cod larvae, however, it remains unclear at what precise combination of temperature and food concentration the switch would occur; our model will remain poorly constrained for many critical parameters until further laboratory experiments on the effects of temperature on larval physiology are conducted.

While our previous choice of Q_a may seem unrealistically high when considering the effect of temperature on assimilation rate, our Q_a term is meant to reflect a suite of other unknown factors besides general assimilation efficiency, which most likely also increase as the temperature increases. These include the swimming speed of the fish, the response and attack speed of the fish during captures, and potentially even the visual acuity of the fish. In any case, it is clear that laboratory experiments investigating temperature effects on larval Atlantic cod and haddock, specifically, are desperately needed to clear up these issues as well. Additionally, in order to achieve realistic growth, we had to reduce the maximum allowed growth rate of our larvae to compensate for the increase in assimilation efficiency that was suggested by Buckley et al., indicating that the actual assimilation efficiency of the fish may be lower than suggested by extrapolation from other fish species. Thus, determining the assimilation efficiency of larval Atlantic cod and haddock, specifically, is another area that deserves investigation.

While the above reexamination focuses on the responses of larval growth to steady forcing, another important point to consider is what happens when the model is forced with a temperature cycle, as was done in Leising and Franks (1999). In that model, the seasonal cycle varied from temperatures near the maximum growth temperature to temperatures near the lower tolerable limit of 4°C (Laurence 1978), depending on the location on Georges Bank. While many of the specific effects of temperature on the assimilation rate, metabolic rate, etc., remain unclear, it is known that these larvae become very stressed at temperatures of 4°C or lower, develop slower (Hunt von Hebring et al. 1996), and usually die if kept at this temperature for too long (Laurence 1978). This presents the growing larvae with a sort of developmental "bottleneck" in terms of the temperature cycle. Thus, increasing the temperature of the *entire cycle* could eliminate this temperature bottleneck at locations where the winter temperature minimum falls to near 4°C and occurs within 40 days of hatching, a conclusion that is independent of our model result and analysis. In conclusion, our statement that "increasing the average temperature cycle by one degree may improve the growth of larvae that were not growing well before" remains justified in light of both this point and our above reanalysis using Buckley et al'.s suggested parameterization.

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		DISCUSSION
Lawrence J. Buckley, R. Gregory Lough, Myron A. Peck, and Francisco E. Werner	**1957–1960**	Comment: Larval Atlantic cod and haddock growth models, metabolism, ingestion, and temperature effects
Andrew W. Leising and Peter J.S. Franks	**1961–1963**	Reply to Buckley et al.'s "Comment: Larval Atlantic cod and haddock growth models, metabolism, ingestion, and temperature effects"

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ARTICLES

N.L. Shackell and K.T. Frank	**1747–1760**	Larval fish diversity on the Scotian Shelf
Ian R. Bradbury, Paul V.R. Snelgrove, and Sandra Fraser	**1761–1772**	Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland
Karen M. Buzby and Sue A. Perry	**1773–1783**	Modeling the potential effects of climate change on leaf pack processing in central Appalachian streams
S. Langitoto Helu, David B. Sampson, and Yanshui Yin	**1784–1793**	Application of statistical model selection criteria to the Stock Synthesis assessment program
Gro I. van der Meeren	**1794–1803**	Predation on hatchery-reared lobsters released in the wild
Guillermo Roberto Giannico	**1804–1813**	Habitat selection by juvenile coho salmon in response to food and woody debris manipulations in suburban and rural stream sections
H. Takahashi and K. Takata	**1814–1833**	Multiple lineages of the mitochondrial DNA introgression from *Pungitius pungitius* (L.) to *Pungitius tymensis* (Nikolsky)
William L. Thompson and Danny C. Lee	**1834–1842**	Modeling relationships between landscape-level attributes and snorkel counts of chinook salmon and steelhead parr in Idaho
Ian D. Hogg, Yves de Lafontaine, and John M. Eadie	**1843–1852**	Genotypic variation among *Gammarus fasciatus* (Crustacea: Amphipoda) from the Great Lakes – St. Lawrence River: implications for the conservation of widespread freshwater invertebrates
Diane Lavoie, Yvan Simard, and François J. Saucier	**1853–1869**	Aggregation and dispersion of krill at channel heads and shelf edges: the dynamics in the Saguenay – St. Lawrence Marine Park
G.T. Kellison, D.B. Eggleston, and J.S. Burke	**1870–1877**	Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*)
D.G. Chen, N.B. Hargreaves, D.M. Ware, and Y. Liu	**1878–1887**	A fuzzy logic model with genetic algorithm for analyzing fish stock–recruitment relationships

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